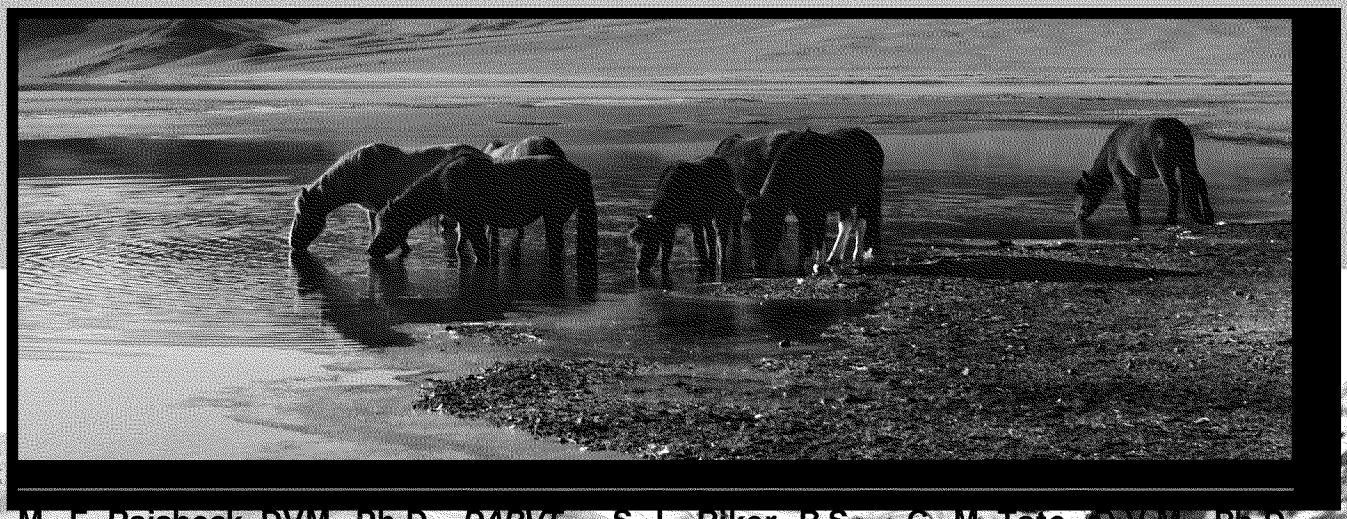
Water Quality for Wyoming Livestock & Wildlife

A Review of the Literature Pertaining to Health Effects of Inorganic Contaminants



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B-1183

Acknowledgements

The authors would like to thank the Wyoming Department of Environmental Quality for providing the funding needed to complete the report and Louise Smithson and Rebecca Dailey from the Wyoming State Veterinary Laboratory and Robert Waggener, editor in the UW College of Agriculture, for the many hours they invested in editing. We thank Bernadette van der Vliet, graphic designer in the College of Agriculture, for her work on layout and design. We would also like to acknowledge the following colleagues who reviewed the report and offered constructive criticism and suggestions.

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Abbreviations

ADG – average daily gain

As – arsenic

Ba – barium

BW – body weight

C – Celsius

CNS – central nervous system

Cp – ceruloplasmin

Cu – copper

DM – dry matter

DMA – dimethylarsonous acid

DMI – dry matter intake

ECF – extracellular fluid

ECG – electrocardiogram

EPA – U.S. Environmental Protection Agency

EU – European Union

F – Fahrenheit

F – fluorine

FDA – U.S. Food and Drug Administration

g – gram

G.I. tract – gastrointestinal tract

GSH – glutathione

Hb – hemoglobin

HDL – high-density lipoprotein

Hg – mercury

H_2S – hydrogen sulfide

IV – intravenous

kg – kilogram

L – liter

lb – pound

LD_{50} – Dose which is lethal in 50% of the tested population

LD_0 – Largest non-lethal dose in tested population (theoretical)

meq – milliequivalent

MetHb – methemoglobin

mg – milligram

mM – millimole

MMA – monomethylarsonous acid

Mo – molybdenum

MT – metallothionein

N – nitrogen

Na – sodium

nAChR – nicotinic acetylcholine receptor

NaCl – sodium chloride

NOAEL – no observed adverse effect level

NO_3^- – nitrate

NO_2^- – nitriteNRC – National Research Council

PEM – polioencephalomalacia

ppm – parts per million

ppb – parts per billion

% – percent

ROS – reactive oxygen species

S – sulfur

Se – selenium

$SOLD_{50}$ – single oral median lethal dose

$T_{1/2}$ – biological half-life

TCA – trichloroacetic acid

TDS – total dissolved solids

TM – thiomolybdates

USDA – U.S. Department of Agriculture

WSVL – Wyoming State Veterinary Laboratory

Contents

1 Introduction

Water is the single most important nutrient for livestock and big game wildlife species. It is the most abundant ingredient of the animal body in all phases of growth and development. A calf's body contains 75 to 80% water at birth and about 55 to 65% water at maturity. While animals can survive for a week or more without food, death is likely in a matter of days without adequate water intake. Water is involved either directly or indirectly in virtually every physiologic process essential to life. Water is the medium in which all chemical reactions in the body take place. Blood, which contains 80% water, is vital in transporting oxygen to the tissues and carbon dioxide from the tissues as well as being the life support system for the body. It is the medium for transporting nutrients, metabolic wastes, and chemical messengers, such as hormones, throughout the body. It provides the chemical base for nutrient digestion and uptake from the GI tract and for the elimination of waste products via urine and bile. Water's physical properties make it an important factor in the transfer of heat and the regulation of temperature in the body. Due to its high specific heat (the ability to absorb or give off heat with a relatively small change in temperature), water is ideally suited as a temperature buffering system for the body. A restriction of water intake lowers feed intake and N retention (i.e. protein), and it increases N loss in the feces. It also results in increased excretion of urea in the urine. Animals may survive a loss of nearly all the fat and about one-half of bodily protein, but a loss of about one-tenth of water from the body results in death.

Obviously, an adequate supply of clean water is necessary to the health of all animals, including human beings. Under most management systems, water is the cheapest and most readily available nutrient. Unfortunately, and probably because of this fact, it is also the most overlooked nutrient. Sources of water include those obtained from wells or surface runoff, water contained in feedstuffs (lush grass may consist of as much as 75% water) and metabolic water obtained from the oxidation of fat and protein in the body. In the arid western United States, good quality water is a scarce commodity, and livestock and wildlife are often forced to survive on what might be charitably described as "less-than-perfect" water due to competition from urbanization, mineral extraction, etc. In most cases, these animals do surprisingly well, but poor quality water has resulted in acute illness and death. It also robs producers via decreased performance (growth, reproduction). Thus, awareness of water quality issues has increased as the competition for resources intensifies. Ranchers, wildlife managers, conservationists, veterinarians, cooperative extension personnel, animal owners, and others need to know whether a particular source of water is safe. One of the more common questions fielded by our laboratories is "what is X ppm of Y in the water going to do to my cattle (horses, deer, etc.)?"

Water consumption is influenced by many factors, including genetics (species, breed), age, body size, ambient temperature and humidity, water temperature, and level of production. For example, cattle (a species that has been studied extensively) consume *an average* of 2 to 4 kg of water for each kg of dry matter consumed and an additional 3 to 5 kg of water per kg of milk produced; however, this average varies dramatically with temperature, especially when the environmental temperature exceeds the thermo-neutral range (5-20 C in cattle) making animals lose increasing amounts of water via respiration and sweating. For example, a 273-kg (600 lb) feeder steer drinks 22.7 L at 5 C or below; at 21 C (70 F), he needs 33 L but at 30 C (roughly 86 F) he requires 54 L or 20% of his body weight per day.[1] At 39 C (roughly 102 F), he would require 116 L.[2] Rations high in Na, fiber, or protein also increase water requirements.[1,3] For example, horses consume twice as much water while on a hay diet compared to a high concentrate diet at the same temperature.[4] The level of production is a very important factor in water requirements. A lactating beef cow requires nearly twice as much water (64 L or about 16% of her body weight) per day at 21 C as the same cow (32.9 L, 9% body weight) when dry (not lactating) at the same temperature, and a high-producing dairy cow of similar size needs 90 L, or nearly 20% of her body weight under the same conditions.[1,5] At 32 C, she may drink as much as 40% of her body weight.[1,6]

The amount (dose) of any water-borne toxicant ingested by a given animal is determined by the concentration of the substance in water *and* by the amount of water the

animal drinks. Water intake is technically defined as free-drinking water plus the amount contained in feedstuffs; however, for purposes of simplicity in this report, we have assumed animals are consuming air-dried hay or senescent forage with a minimal (10%) water content and will use the term "intake" to describe the amount of water consumed voluntarily by animals from streams, ponds, etc. The amount an animal drinks is determined by true thirst and appetite. By definition, true thirst is the physiologic drive to consume sufficient water to meet minimum metabolic needs; however, most animals also exhibit an "appetite" for water and consume more than is strictly necessarily to satisfy thirst.[7] Reasons for the latter are many, varied, and do not lend themselves to quantitative prediction. We therefore disregarded appetite in calculating doses from water intake but instead used fairly conservative estimates of thirst in such calculations by disregarding forage water content. Most calculations of potential toxic doses in this report are thus based upon 273 kg (600 lb) feeder cattle that drink approximately 20% of their body weight, or about 8 L per kg of dietary dry matter, per day, at 32 C (90 F). This *may* not provide adequate protection for high-producing dairy cattle, which drink significantly more under similar environmental conditions, but is reasonably conservative for range livestock (beef and sheep) and weather conditions typical of Wyoming. Higher temperatures would also result in higher consumption than our "standard" steer, but sustained periods of such weather are not that common in Wyoming.[8] Finally, there is virtually no information on water consumption by the major wildlife species covered in this report, but it is reasonable to assume that species that evolved in the northern Great Plains would not have greater requirements than domestic cattle.

This report is targeted at domestic livestock and wildlife (beef cattle, horses, sheep, deer, elk, and pronghorn antelope) that rely upon wells, ponds, streams, and other water sources on Wyoming's ranges. Although we have made note of data related to swine where we found them, virtually all modern swine are raised in intensive operations that draw water from systems (municipal, water district, etc.) that are maintained according to human drinking water standards. Similarly, "alternative agricultural" species such as llamas and bison are not included, in part because of a scarcity of data.

Water quality is commonly evaluated by chemical methods that have been designed to be very reproducible and very specific. As a result, the process of analyzing water is fairly straightforward, and many tests are readily available, commercially. Unfortunately, translating these very precise, formal, data to practical recommendations for livestock and wildlife is less cut and dried. As noted by Dr. Art Case, the dean of veterinary toxicologists, "sometimes the cow just didn't read the book." First, many toxicants in water act additively with the same toxicant in feedstuffs. In most such cases, the bottom line is not necessarily the water concentration but rather the total mg of toxicant ingested per kg of the animal's body weight (commonly expressed as "mg X/kg BW"). Throughout this report, we have tried to use realistic estimates of total dietary concentrations of such toxicants to calculate the water concentration of the toxicant required to potentially cause problems.

Second, chemical water quality tests do not usually measure the specific chemical form of the toxicant present. For example, Se as selenite or selenate behaves quite differently in the mammalian body than does selenomethionine, but the typical laboratory just reports total Se. Where possible, we have based recommendations upon the chemical form most likely to be present in typical surface waters in Wyoming and noted any caveats that should be considered if the water source is not "typical". In the absence of other data, we have assumed the free ion in water is equivalent on a mg/kg BW basis to the same chemical in feedstuffs.

Third, typical chemical tests do not differentiate between animal species. Some substances are more toxic in ruminants than monogastrics (simple-stomached animals) as a result of their unique physiology; some are less. While we have tried to identify significant differences where they exist, our recommendations are based upon the most sensitive of our species of interest.

Fourth, many toxic substances interact with other toxicants and/or nutrients in the diet. We have tried to enumerate such interactions in the narrative if they are well documented and, where possible, account for them in the "bottom line" calculations of acceptable water concentrations.

Finally, the rate of exposure influences the potency of many toxicants. A bolus dose of nitrate (NO_3), given via a stomach tube, is much more toxic than the same amount spread over an entire day's grazing. Under summer range conditions typical of the Great Plains, livestock drink once, or, at most, twice a day. Wildlife typically trek to water and drink their fill during the morning and evening twilight. We have, therefore, assumed all of the water-borne daily dose of a given

substance will be consumed during a fairly short period, once or twice a day.

Water quality constituents in this report were drawn from common water quality guidelines, prioritized according to how closely, in our experience, existing Wyoming concentrations approached these guidelines and how often the elements in question caused poisoning in Wyoming animals. For example, Hg is much more toxic than many of the elements we studied, but it is rarely present at detectable concentrations in Wyoming water surveys. Copper is a real problem in aquatic organisms, but Cu deficiency is a much bigger problem in livestock than Cu toxicity. We then worked our way as far down this prioritized list as time permitted. Obviously, there are more constituents on our list than we were able to examine, but we believe we covered those most important to Wyoming.

Data used in compiling this report are drawn primarily from scientific literature, including refereed journals, texts, proceedings, abstracts, and theses, with an emphasis on material published during the last 20 years. The basic strategy consisted of 1) searching biomedical databases (e.g. Medline, CAB Abstracts, etc.) for reports of toxicity in any species, 2) examining bibliographies of relevant papers for new leads, and, finally 3) forward searching (e.g. Science Citation Index) for more recent papers that cite earlier work on a given topic. We also solicited well-documented anecdotal data (i.e. field reports) from colleagues at other research and/or diagnostic institutions. Where possible, we tried to validate secondary sources (e.g. reviews, texts) by examining primary documents from which they were drawn. If sufficient data existed for our principle species of interest (beef cattle, horses, sheep, elk, deer, and antelope) we focused on those reports. If not, we attempted to extrapolate from rodents, humans, etc., being careful to identify the uncertainty factors inherent in such extrapolations. Each source was assigned a rating for reliability, with peer-reviewed, experimental studies usually, but not always, being considered more reliable.

As noted previously, the interaction of water quality and animal health is considerably more complex than just "X" mg of "Y" per L of water. For example, many factors have been suggested to influence the palatability of water for animals. Decreased consumption due to bad taste is potentially just as harmful as water deprivation[3], yet the state of the art regarding palatability is still largely qualitative and anecdotal. Acid pH may mobilize toxic metals from plumbing or soil, but the particular effect of a given pH is obviously very dependent on the local situation. A sudden transition to pure water after several weeks on highly saline water may result in so-called "salt poisoning." Where adequate, quantitative data exists for non-directly toxic adverse effects on health, we have incorporated them into the final recommendations. Where there is substantial evidence suggesting such effects exist, but no reproducible, quantitative data were available, we tried to mention the existence of such effects but have not factored them into the final recommendations.

Safety margins are a matter of judgment rather than an exact science. The purpose of safety margins is to compensate for unknown, or unknowable, variables in toxicology data such as genetic variability, sex, life stage, duration of exposure, unforeseen interactions with other toxicants, etc. The standard practice in setting human drinking water standards for non-carcinogens has been to divide the geometric mean of the NOAEL and minimum toxic dose by 10 to 1,000 depending upon whether the data are derived from human exposure, multiple non-human species, or incomplete data in any species. Another approach used in the past has been to set the safe limit at the upper end of the range commonly reported in natural waters as was done with Se.[2] Both approaches, while unarguably "safe," ignore the realities of livestock production in the western United States. Water that is so "perfect" as to meet these theoretically desirable criteria has already been taken for other uses. In this report we have taken the approach of presenting our best estimate of the NOAEL (i.e. will not produce any measurable decrease in performance in the most sensitive class of animal) under a very conservative set of assumptions appropriate to Wyoming and allowing readers to make their own judgment regarding "safety" margins.

The final report, together with the documents it was drawn from, was forwarded to colleagues at four other universities (Washington State University, University of Nebraska, North Dakota State University, and Texas A&M University) for peer review. Their comments were considered and incorporated into the final document.

Although there are many ways of expressing measurements regarding water quality and toxicology, we have chosen to use the following conventions. The dose of a toxicant that causes some particular effect is expressed in milligrams of substance per kilogram of body weight or "mg X/kg BW". The concentration of a substance in water is expressed as milligrams of substance per liter of wa-

ter or "mg X/L". If the substance is ionized, and the ion is important in terms of toxic effects, it will be described with the standard scientific abbreviation for the ion, e.g. "NO_3^-". Similarly the concentration of a toxicant occurring in dry feedstuffs will be described in terms of parts per million or ppm. Single elements are abbreviated with the standard chemical symbol (e.g. "Se" for selenium).

This report, and the project that created it, was funded by the Wyoming Department of Environmental Quality. Although the authors anticipate they will find the information useful, our intended audience is much broader and includes ranchers, wildlife managers, conservationists, veterinarians, cooperative extension personnel, animal owners, and others. The last concerted effort in the United States to summarize the literature regarding water quality for animals occurred more than 30 years ago[2], and there have been many additions to the knowledge base since that time. We believe this report represents a reasonable starting point for evaluating the adequacy of water quality for animals.

2 Arsenic

Arsenic (As) is a metalloid that occurs naturally in water and soil. It is also released from a number of human activities including mining, petroleum and natural gas extraction, wood preservation, and burning coal.[9-16] Although As is rare in nature as a pure element, both inorganic and organic forms of As are commonly found in a number of different oxidation states, of which two (+3 and +5) occur in soil, water, and vegetation. Inorganic forms of As^{III} (e.g. the arsenite ion) may be found under reducing conditions; however, As^{V} (e.g. the arsenate ion) predominates in surface waters containing considerable dissolved oxygen.[17] Organic arsenical compounds have also been used as herbicides, insecticides, and drugs. Arsenic was one of the first drugs to be used successfully against the syphilis organism in humans. The organic arsenical roxarsone (4-hydroxy-3-nitrobenzenearsonic acid) is still used to control coccidia in poultry and swine. More recently, arsenic trioxide (As_2O_3) has been suggested for treatment of promyelocytic leukemia and multiple myeloma.[18]

Most of what was known about the toxicity of As prior to the 1980s was based upon the direct cytotoxic effects of As.[19] More recently, however, chronic consumption of low-level As-contaminated drinking water has become associated with a variety of chronic maladies in human beings, including cardiovascular disease, "blackfoot disease," diabetes mellitus, spontaneous abortion, and, especially, cancer.[9,20-22] Conversely, some reports[23,24] suggest a hormetic (beneficial) effect of As at very low doses. So far the majority of the evidence for chronic effects in humans consists of epidemiological studies, but mechanistic, toxicologic explanations are appearing in the literature. The latter are important because they suggest these effects (and therefore dosages) are not likely relevant to our species of interest (see below for details).

The nomenclature of the arsenicals is often bewildering. In this text, the following conventions are used: the trivalent, methylated metabolites (mono)methylarsonous acid, and dimethylarsonous acid will be abbreviated as MMA^{III} and DMA^{III}, the pentavalent metabolites (mono) methylarsonic acid, and dimethylarsinic acid as MMA^{V} and DMA^{V}, and the inorganic ions such as arsenite and arsenate as iAs^{III} and iAs^{V}.

Essentiality

Although the physiological function(s) of As remains unknown, experiments with As deprivation in many species suggest As may be an essential element.[25] For example, rats maintained on a diet containing 30 ppb As exhibited decreased growth rate, rough hair coats, and decreased hematocrit levels when compared to controls supplemented with 4.5 ppm As.[26] Lactating goats consuming a diet containing less than 10 ppb As had decreased growth rates, decreased milk production, lower birth weights, and a higher incidence of mortality.[27] Female miniature pigs on a low As diet produced smaller piglets, and only 62% of pigs were reported to give birth when compared to pigs supplemented with 350 ppb As.[27] The results of experimentally induced deprivation in both *in vivo* and *in vitro* experiments suggest As plays a role in the methylation of both proteins and genetic molecules.[25] Although this and other, similar research is scientifically interesting, As deficiency has never been demonstrated in nature, probably because the apparent nutritional requirements are considerably lower than common background concentrations.

Metabolism

It is commonly accepted that, with the possible exception of the trivalent methylated metabolites (MMA^{III} and DMA^{III}), the inorganic forms of As are considerably more toxic in mammals than organic arsenicals.[20,25,28-30] Since the inorganic forms are also by far the most common contaminants of water under field conditions and because MMA^{III} and DMA^{III} are too unstable to persist for any length of time under natural conditions, this review will focus upon exposure to the inorganic forms of As^{III} and As^{V}. Arsenate is absorbed from the gut by a two-stage process. First, it is concentrated in mucosal cells then, as binding sites become filled, it moves down the resulting concentration gradient into the portal circulation.[25] The absorption mechanism of iAs^{III} is less completely understood, but it is commonly accepted that iAs^{III} is even more readily absorbed than iAs^{V}, probably because of its greater water solubility.[17] Once absorbed, As is transported to various tissues via blood. Distribu-

tion between the erythrocytic and plasma components of blood depends upon the dose of As given, the species of animal, and the valence of administered As. In general plasma concentrations increase relative to red cell concentrations as the dose increases, and trivalent As has a higher affinity for erythrocytes than As^{V}, resulting in slower clearance. Humans, rats, and mice have higher erythrocyte binding than other domestic mammals, also resulting in slower elimination.[17,27]

The metabolism and excretion of As varies significantly between species and between genotypes within the human species.[27,31-33] These species-specific differences in metabolism are important in the pathogenesis of As intoxication in any given species.[17,27] Generally speaking, inorganic As is methylated *in vivo* via a series of sequential reduction and oxidative-methylation reactions. Inorganic As^{V} is reduced in a linked reaction with oxidation of reduced glutathione (GSH) to iAs^{III}, which is then methylated to MMA^{V} by reaction with S-adenysylmethionine. Monomethylarsonic acid (MMA^{V}) is, in turn, reduced and methylated to DMA^{V} and so forth.[22] These metabolites are more readily excreted than inorganic As. In many, but not all, mammalian species, this process proceeds to DMA^{V} or the trimethyl arsenic metabolite trimethylarsine oxide (TMAO); however, in human beings, significant amounts of MMA^{III} and DMA^{III} apparently escape methylation and react with critical tissue components. The rate of methylation and physiologic site of metabolism are thus important determinants of the rate of elimination and the potential for chronic effects at very low doses.[22,29]

As interacts with many other dietary factors, resulting in either increased or decreased toxicity. As has been known to minimize the toxicity of Se for many years.[34-37] When As is fed with Se, the excretion of both elements in feces is enhanced.[37,38] Simultaneous administration of Zn lessened the toxicity of As-spiked drinking water, possibly by induction of metallothionein, a metal binding protein.[22,39] Folate can affect As methylation and both folate deficiency and excess As induce fetal malformations in rats, thus a dietary deficiency of folate potentiates As toxicity.[22,40]

Toxicity

The acute toxicity of inorganic As has been attributed to the generation of reactive oxygen species (ROS) and oxidative stress[18,21,39], to the denaturation of critical protein moieties, and to interfering with phosphate metabolism.[17,41] In virtually all models, iAs^{III} is reported to be several fold more toxic than iAs^{V}.[28,30,41,42] Trivalent iAs^{III} exerts its toxic effects by binding with specific functional groups (thiols and vicinyl sulfhydryls) on enzymes, receptor proteins, etc.[10,17,27,43] For example, mitochondrial respiration is blocked by the reaction between As^{III} and the dihydrolipoic acid cofactor required for substrate oxidation.[41] As cited by Thomas et al.[44], in 1966 Webb listed more than 100 enzymes that were inhibited by As^{III}. Pentavalent As^{V}, because of its chemical similarity with the phosphate ion, displaces phosphate from certain biochemical reactions, e.g. oxidative phosphorylation, resulting in depletion of ATP[10,41,43-45], but it may also be converted to As^{III} *in vivo* (i.e. in tissues) and in the rumen.[45] The clinical signs associated with acute toxicity in virtually all species include diarrhea, vomiting, abdominal pain, weakness, staggering gait, myocardial degeneration, and, in some cases, death.[17,25,30,43,45]

Most acute As toxicity results from accidental exposure as a result of improper handling, use, and/or storage of arsenical compounds.[13,15,45] Cattle began staggering and showing signs of abdominal pain, diarrhea, anorexia, and recumbency shortly after ingesting forage that had been sprayed with sodium arsenite ($NaAsO_2$). Analysis of the grass performed several days later revealed it contained 2 ppm As, but the author noted concentrations were probably higher immediately after spraying.[46] Cows and calves became recumbent and exhibited a rapid weak pulse, intermittent clonic convulsions, and paddling movements after drinking fluid from a dipping vat containing 200 mg As/L.[47] A herd of approximately 275 cattle allowed to graze a road right of way recently sprayed with sodium arsenate (Na_2HAsO_4) sickened within hours, and 80 died within four days. Samples of grass taken at the onset contained 10,500 ppm As.[14] Selby et al. reported a similar scenario in Missouri in which the toxic vegetation contained 440 ppm As.[48] Cattle that consumed water containing 6-20 mg As/L and silage contaminated with 140 ppm As became progressively weaker, emaciated, and recumbent, had decreased milk production, and eventually died after several days.[49] Nine adult cattle developed acute hemorrhagic diarrhea, and two eventually died after consuming a dairy premix containing 5.50 ppm As; however, the As concentration of G.I. contents suggests the exposure may have been considerably higher.[50] Heifers became weak, recumbent, dysenteric, and died after ingesting vegetation containing approximately 2,000 ppm As from herbicide contamination.[51] Approximately 12 hours after consuming pellets containing 27,000

ppm As and 20 ppm metaldehyde, cattle developed ataxia, profuse diarrhea, and muscle fasciculations.[52] The authors suggested that most of the toxic effects were due to As because the predominate clinical signs did not fit metaldehyde.

The toxicity of the herbicide, lead arsenate ($PbHAsO_4$), is thought to be due to the As content rather than Pb.[53-55] After licking bags containing $PbHAsO_4$, cattle exhibited rapid pulse and respiration, oral mucosal erosions, diarrhea, and decreased milk production. Arsenic toxicity was confirmed when stomach contents were found to have 175 ppm As.[53] Yearling cattle exhibited severe colic, diarrhea, and death after consuming an undetermined amount of a powder containing 39% Pb and 10% As. Rumen contents from the animals contained 478-531 ppm As.[56] Five calves began showing signs of lethargy, ataxia, anorexia, decreased heart rates, and diarrhea after consuming powder containing 700,000 ppm As_2O_3. Four of the animals eventually died.[57] Cattle receiving 67 g As_2O_3 and calves receiving 17 g As_2O_3 as a topically applied medication became depressed and exhibited bloody diarrhea and a staggering gait soon after treatment. By 20 hours post treatment, 94 of 101 animals were dead.[58] Depression, ataxia, weakness, recumbency, diarrhea, abdominal pain, and tachycardia developed in 15 cattle that ingested ash from As-preserved wood. The ash was found to contain 780 ppm As.[59] After 260 heifers were moved to a new pasture containing an abandoned cattle dip, they became restless and belligerent and began showing signs of profuse salivation and watery diarrhea. (Watery diarrhea is extremely dilute and may even be clear; it's usually a result of runaway secretory processes in the bowel.) More than 50% of the herd exhibited convulsions and became comatose, and 67 eventually died. The soil around the dip contained 10-150 ppm As and 50-500 ppm toxaphene. The authors suggested that, because tissue As concentrations were not diagnostically significant, the combination of As and toxaphene caused the die-off.[60] Experimentally, four of five cattle died within 10 days of being dosed with 10 mg As^V/kg BW/day as monosodium methanearsonate.[61]

More than 650 of 1,000 sheep developed diarrhea and died after consuming vegetation that had been sprayed with a $PbHAsO_4$ solution containing 0.58 % As.[62] The condition was reproduced by dosing lambs with 12 mg As/kg BW.[62] Lambs showed signs of depression, abdominal pain, salivation, and diarrhea after ingesting forage containing 62-95 ppm As due to contamination with a cotton defoliant; 172 of 923 lambs died.[63] Six doe white-tailed deer were found dead after eating soil and vegetation contaminated by aerial spraying of an arsenical herbicide at the rate of 1.6 lbs As per acre. Later analysis of a combined soil and vegetation sample yielded 2.4 ppm As, and water samples yielded 0.36-0.48 mg As/L.[64] The application rate, however, calculates to a forage concentration of approximately 368 ppm or a dosage of approximately 11 mg As/kg BW in an animal consuming 3 % BW daily. The latter numbers are more consistent with reported tissue concentrations (18-19 ppm As) in the dead deer than the soil and water analysis.

Arsenic poisoning has also been reported in monogastrics. Nine thoroughbred racehorses died after showing signs of extreme distress, weakness, colic, rapid, weak pulse, hyperemic mucous membranes, and watery diarrhea. It was discovered that roughly 8 oz. of arsenical rat poison had spilled into their corn bin. *Post mortem* chemical analysis discovered significant amounts of As in the stomach and liver of two horses.[65] Working backward from the numbers presented, we estimate the horses received a dose of between 1-10 mg As/kg BW. Gastrointestinal cramps, vomiting, diarrhea, ECG changes, and liver disruption developed in a 27-year-old woman after she ingested 9,000 mg As_2O_3.[66] Furr and Buck[67] poisoned cats with a commercial ant bait. Doses of As (as Na_2HAsO_4) greater than 8 mg/kg BW were lethal; the threshold of toxic signs was 2 mg/kg BW, and the no-effect level was 1.5 mg/kg BW.

In swine, arsanilic acid has been frequently used as a growth promotant and as a treatment for swine dysentery. In several cases, excessive doses or prolonged treatment periods have resulted in a chronic syndrome characterized by apparent blindness due to degeneration of the optic nerve and optic chiasma.[68-74] The toxic mechanism and toxicity of this class of arsenical drugs are quite distinct from and less than inorganic As and will therefore not be considered further.

At present, despite convincing epidemiologic evidence that very low concentrations of As in drinking water can cause chronic disease, especially cancer, in human beings, there are no animal models that reliably duplicate these particular toxic effects without resorting to relatively high doses and/or pharmacologic and genetic manipulation to render them more sensitive.[19,33] The current belief, derived from *in vitro* studies and specialized laboratory animal models, is that small amounts of DMA^{III} and MMA^{III} escape the methylation process in people and, over prolonged periods, cause cellular damage that results

in diseases such as cancer.[10,20,23,32,33,41,44] Dimethylarsinic acid (DMA^V) and monomethylarsonoic acid (MMA^V) are the main urinary metabolites of As excreted in most mammals; however, the trivalent As metabolites, dimethylarsinous acid (DMA^{III}), and monomethylarsonous acid (MMA^{III}) have been discovered in fresh urine of As-poisoned human patients.[20] The glutathione conjugate of DMA^{III} was actually more toxic to cells *in vitro* than inorganic As[20], and DMA^{III} causes single-strand breaks in DNA *in vitro*.[20,75] These processes are apparently limited to human beings and specialized laboratory models; thus, based upon known differences in metabolism, this class of disease and dosages does not seem relevant for livestock and big game animals.

Developmental studies of orally administered MMA^V and DMA^V (the metabolites of inorganic As in most non-human mammals) in rats and rabbits determined the threshold of fetal damage was similar to that for maternal toxicity or about 36 and 48 mg/kg BW, respectively.[76] Administration of As by gavage to pregnant rats and mice did not produce morphologically evident teratogenesis at non-maternally toxic dosages, although there was some evidence of behavioral changes in pups born to dams consuming drinking water with slightly less As^{III} than the lowest maternally toxic dose.[22] Conversely, Domingo[77] reported that As^{III}, via the oral route of exposure, was much less teratogenic in several species. This indicates that dietary limits safe for a dam should also provide adequate protection for her fetus.

There are very few reports of chronic toxicity in non-rodent animals. Due to the rapid excretion of As in cattle, sheep, dogs, etc., these species are able to clear less-than-acutely-toxic doses of As before they can cause much of a problem.[25,78] The reports of chronic toxicity we discovered involved dosages similar to those reported for acute or subacute poisoning. Female beagle dogs fed 4 to 8 mg $NaAsO_2$ (2.3-4.6 mg As)/kg BW per day for 183 days exhibited decreased weight gains due to decreased feed consumption and slightly elevated liver enzymes.[79] Beagle dogs were fed varying concentrations of As as either As^{III} or As^V for two years. At 50 ppm and less, there were no measurable effects. At 125 ppm dietary As, the dogs lost weight and several died with lesions of inanition.[80] Three of four sheep given 88 mg $PbHAsO_4$/kg BW once per month died within 24 hours of the seventh dose. The other sheep in the study, given 22 and 44 mg $PbHAsO_4$ (4.9 and 9.7 mg As/kg BW), survived 11 doses with no clinical signs.[54] One of two lambs dosed with 1.5 mg As/kg BW/day as $PbHAsO_4$ died after 35 days; the other survived until the study was stopped at 94 days.[62] Sheep fed a mean daily dose of 1.4 mg As/kg (As species unspecified) for three weeks remained in good condition for the duration of the study.[81] Bucy et al.[82] fed potassium arsenite to feedlot lambs at doses as high as 3.26 mg As/kg BW/day for eight weeks without adverse effects. In a later study 1.75 mg/kg BW/day was not toxic, but higher doses caused feed refusal and clinical signs of toxicity.[83] Peoples[78] added arsenic acid to dairy rations at 1.25 ppm, a dose of approximately 0.48 mg As/kg BW/day for seven weeks with no effects. Virtually all of the ingested As was eliminated as quickly as it was eaten.

It has been proposed[84] that elk in the Madison-Firehole watershed of Yellowstone National Park have shorter lifespans because of naturally elevated As in water and feedstuffs. Exposure was estimated to be "greater than 1.25 mg/kg BW/day" based upon tissue concentrations and extrapolation from bovine studies and between "0.01 - 6.2 mg/kg BW/day" based upon forage and water analysis. The difference in longevity also may be due to other environmental differences between the Madison-Firehole area and the control site.[84] Forsberg et al.[85] demonstrated slight but measurable inhibition of normal rumen fermentation *in vitro* with As concentrations as low as 5 mg/L of rumen fluid, but they did not provide any data as to how this concentration related to dietary intake. Assuming for the moment that the rumen fluid concentration is equivalent to the combined concentrations in feed and water, the concentration would be equivalent to a dose of roughly 1 mg As/kg BW.

Summary

Our recommendations are based upon the toxicity of inorganic As^{III}, specifically the arsenite ion. Routine water quality analysis available to livestock producers does not distinguish between As species, and, although the less toxic pentavalent forms of As are more likely to occur in surface waters, trivalent As is seen frequently enough in specialized surveys to justify the assumption.[86,87] It is suggested that ruminant animals are less susceptible to As than monogastrics.[25,88] With the exception of laboratory rodents, however, we were not able to confirm this to be the case, thus we have assumed horses are equally sensitive to As as ruminants.

Chronic poisoning of the type (cancer, "blackfoot disease," etc.) that prompted lowering the human drinking water standard from 0.05 to 0.01 mg As/L does not apparently occur in other animal species, as demonstrated

by the ongoing search for an appropriate animal model to study the human condition. The mechanism(s) putatively involved in the pathogenesis of chronic damage in people, i.e. chemical attack by methylated As^{III} metabolites on cellular macromolecules, do not appear to be relevant in livestock and wildlife. In domestic livestock, as opposed to people, most As is excreted via urine as DMA^{III}.[33] This, together with the shorter observed half-life in these species, suggests that relatively little trivalent As escapes methylation and excretion to cause cancer. Chronic poisoning in livestock species involves mechanisms similar to acute poisoning and requires dosages very similar to acute poisoning.

Given the accumulating evidence that As is a human carcinogen, the question of residues arises. Can food animals consuming As from water accumulate dangerous amounts of As in edible tissues without themselves showing signs of toxicity? The literature to date suggests cattle, sheep, etc. eliminate As too quickly for this to be a concern, and a study completed in 2007 by the University of Minnesota[89,90] failed to find any evidence of As accumulation in milk or edible tissues from dairy cattle watered from As-contaminated (140 µg/L) wells.

The threshold toxic dose in domestic ruminants appears to be between 1-2 mg/kg BW. This dose is in general agreement with the NRC[25], which recommended 30-50 ppm dietary As as a maximum tolerated dose and with other reviews.[88,91-93] It is quite distinct from the EU recommendation of 2 ppm dietary As, for which we have not been able to discover any justification. Sufficient quantitative data was not found to estimate a similar threshold for horses, but this dose is similar to that reported in another monogastric species (dogs)[79], and previous reviews suggest horses are similar to cattle in sensitivity and/or less frequently affected than cattle under similar conditions.[93,94] Therefore, it *seems* reasonable that limits safe for cattle should be adequate for horses. The very limited data in wild ruminants suggest they are similar to cattle in sensitivity. Therefore, our recommendations are based upon dosage data from cattle and sheep. Assuming neglible As in feedstuffs, 5 mg As/L in drinking water will provide the minimum toxic dose of 1 mg As/kg BW to grazing animals in warm weather. Obviously, if animals are receiving any As from forage or medications, less will be required to achieve a toxic dose. Although we were not able to find any significant studies of As in Wyoming forages, limited data from our laboratories suggest natural background concentrations seldom exceed a few ppm, except in areas contaminated by geothermal runoff.

Assuming a NOAEL of 0.5 mg/kg BW/day and allowing for these small forage concentrations, *we recommend drinking water for livestock and wildlife not exceed 1 mg As/L*.

3 Barium

Barium (Ba), an alkaline earth element, oxidizes easily when exposed to air, and it is found as the Ba^{2+} ion in water. Barium found in surface and ground waters is predominantly derived from weathered rock and minerals. Common naturally occurring Ba minerals are insoluble barite (barium sulfate, $BaSO_4$) and somewhat more soluble witherite (barium carbonate, $BaCO_3$), while the Ba^{2+} ion is most common in natural waters.[95] Barium concentrations in water will likely be higher near drilling platforms than natural background concentrations as a result of drilling muds, cuttings, and produced water discharge containing Ba.[96] In water, soluble Ba may precipitate out of aqueous solution as insoluble salts (e.g. $BaSO_4$ and $BaCO_3$). At pH activity of 9.3 or below, the formation of $BaSO_4$ limits the Ba concentration in natural waters. Barium has a variety of uses: $BaSO_4$ is used in patients for digestive tract imaging and for oil drilling, and $BaCO_3$ is used in rodenticides.[96-98]

Essentiality

Barium is not an essential element for plants or animals.

Metabolism

Existing studies indicate Ba absorbed from the G.I. tract is primarily deposited in bones and teeth and excreted via feces and urine.[99-103] The absorption efficiency of various Ba compounds given orally varies widely (0.7-85%) depending upon the chemical form, species, age, and fasting status of the animal.[104-106] In general, more soluble forms of Ba such as barium chloride ($BaCl_2$) are more readily absorbed. Young rats absorb approximately 10 fold more $BaCl_2$ than adults.[106] Barium disappears from blood and milk with a half-life ($t_{1/2}$) measured in days;[100,102] however, Ba deposited in bone has a $t_{1/2}$ measured in years, and disappearance from bone is generally dependent upon bone turnover.[107] These observations, together with the divalent cationic nature of Ba and the fact Ba is known to bind Ca-dependent enzyme systems in cells, suggest Ba metabolism utilizes Ca transport systems in the body.

Toxicity

Barium is toxic in water-soluble forms such as $BaCl_2$ and, to a lesser extent, $BaCO_3$. Barium sulfate is insoluble and is not considered hazardous to people or other monogastric animals. The specific toxic mechanism of Ba is a blockade of passive transmembrane potassium (K^+) conductance in excitable cells by the Ba^{2+} ion.[97,98,108,109] Barium also competes with and/or mimics the functions of Ca in muscle contraction and in second messenger pathways.[97,108] The characteristic systemic effect of Ba poisoning is "violent contraction of smooth, striated, and cardiac muscle."[109,110] Clinically, this effect is manifested as arterial hypertension and premature supraventricular and ventricular contractions, followed by skeletal muscle contraction, salivation, vomiting, colic, and diarrhea.[97,98,108,109,111,112] Subsequently, blood pressure drops precipitously and skeletal muscles exhibit flaccid paralysis. Finally, death results from arrhythmias and cardiac failure.[104,112,113] The hypokalemia seen in Ba poisoning is thought to result from blockade of passive K channels and intracellular sequestration, as Ba has no proven activity on the Na^+K^+ATPase pump.[98,109,114]

Data on the toxicity of Ba in grazing animals is limited. Two ruminally fistulated dairy goats were infused with 5mM $BaCl_2$ at a rate of 60 ml/hr. The ruminal fluid Ba^{2+} ion concentration was estimated to be 0.4 mM assuming no absorption or precipitation occurred. After receiving Ba^{2+} for six hours, the animals exhibited weakness and paralysis, and they died later that night. The resulting oral lethal dose of $BaCl_2$ in the goat was determined to be less than 4.6 mg Ba^{2+}/kg BW.[115] This Ba dosage would equate to approximately 23 mg/L in drinking water under conditions outlined in the Introduction. Ba poisoning occurred during two successive years in cattle that were trailed through an abandoned lead/silver mine site containing a Ba-contaminated pond.[116] Affected animals exhibited protruding tongues, salivation, watery diarrhea, muscle tremors, and paralysis progressing to recumbency and death. Six of 30 animals died the first year and 16 of 20 the second. Liver and kidney tissues from affected animals contained elevated Ba concentrations, and other metals (Pb, As, Se, etc.) were within normal limits. Pond water contained 2.2 mg Ba^{2+}/L; however, clay found in

both the abomasum of the dead cattle and in the pond contained 69,000 ppm Ba. The amount of Ba, if any, absorbed from the clay is presently unknown. X-ray diffraction analysis of the clay indicated the Ba was present primarily as insoluble $BaSO_4$, with lesser amounts of $BaCa[CO_3]_2$ and $BaCO_3$ salts. Reagor[117] fed $BaCO_3$ to steers at 0.4% and 0.8% of the dry matter diet to steers (0.28% and 0.56% Ba respectively). All three receiving the high dose died after a single day on feed; all three receiving the lower concentration remained healthy for the duration of the experiment. Clinical signs in the steers were similar to those reported in monogastrics.

Malhi et al.[118] investigated the optimum concentration of $BaCO_3$ for a rodenticide and concluded 1.5 g of $BaCO_3$ per 100 g body weight (or 15,000 mg/kg) was the most efficient rat poison. There was no attempt to determine a minimum lethal dose. Mattila et al.[119] administered $BaCl_2$ via the marginal ear vein of six rabbits instrumented with an electrocardiograph. Three to 5 mg Ba^{2+}/kg of BW caused dysrhythmias with and without convulsions. Rabbits receiving 3 mg Ba^{2+}/kg of BW survived, despite convulsions. Roza and Berman[112] observed anesthetized dogs after infusing them with 0.66-2.64 µmol Ba (as $BaCl_2$)/kg/min to identify mechanisms of Ba-induced hypokalemia and hypertension and to study the Ba-K interaction on the heart *in vivo*. They found rates greater than 4 µmol $BaCl_2$/kg/min (~362 µg Ba^{2+}/kg/min) were fatal within a few minutes due to respiratory paralysis.

In humans, most acute oral toxicity data is derived from suicide attempts. Gosselin[110] describes the oral lethal dose in humans as "1-15 g." A research chemist attempted suicide by ingesting a teaspoon (approximately 13 g) of $BaCl_2$.[120] He was rushed to a hospital and survived after treatment with $MgSO_4$ and KCl. Seven members of a family were poisoned with Ba after ingesting fried fish accidentally breaded with powdered rat poison.[114] The exact amount of $BaCO_3$ ingested was not determined, but the breading of the fish contained 105,000 ppm of Ba. Three of the family members displayed classic signs of Ba poisoning, while one developed rhabdomyolysis, respiratory failure, and hypophosphatemia. All patients survived with treatment. In another instance, a 26-year-old man consumed one can of "Magic Shave" containing 12.8 g of Ba^{2+} ion and 3 g of sulfide.[121] Ipecac was given within three hours. He suffered respiratory paralysis, severe respiratory acidosis, and hypokalemia. His condition improved after he was given 206 meq of K over 19 hours.

Several studies have examined the chronic oral toxicity of low concentration $BaCl_2$ in drinking water to people because of Ba's recognized cardiac toxicity. Wones et al.[122] concluded that drinking water concentrations of 5 and 10 mg Ba^{2+}/L did not affect any known modifiable cardiovascular risk factors. Eleven healthy men were given $BaCl_2$ in their drinking water for six weeks. For the first two weeks of the experiment, no $BaCl_2$ was added to their water. $BaCl_2$ was then added at rates of 2-5 mg/L for the next four weeks and 10 mg Ba/L for the last four weeks. The subjects were given 1.5 L of treated drinking water per day; any additional drinking water was distilled. Diets were controlled as well as other known causes of cardiovascular disease. Blood (plasma total triglycerides and HDL cholesterol), urine (Na, K, vanillylmandelic acid, and total metanephrines), blood pressure, and cardiac function were monitored throughout the study. They discovered no apparent changes in modifiable cardiovascular risk factors, but there was a trend toward slightly increased total blood Ca. Brenniman and Levy[123] conducted an epidemiological study to determine if mortality and morbidity rate were significantly increased in human populations drinking elevated Ba levels in their drinking water as compared to populations with little or no Ba exposure. Cardiovascular mortality rates were surveyed in communities with drinking water Ba concentrations that ranged between 2-10 mg/L. A morbidity study was also conducted in areas where mean Ba concentrations in drinking water were 0.1 and 7.3 mg/L. They found higher cardiovascular mortality rates in elevated Ba communities, but there were also several confounding variables. There were no significant differences in blood pressure, hypertension, stroke, and heart and kidney disease.

The single oral LD_{50} of $BaCl_2$ in rats was estimated to be approximately 264 mg Ba/kg BW.[124] Short-term (1-10 day) oral exposure to $BaCl_2$ at daily doses up to 138 mg Ba/kg BW produced no significant adverse health effects. Because of the link with cardiovascular disease in people, most chronic laboratory animal studies focus on cardiac effects. Barium acetate ($Ba(CH_3COO)_2$), added to drinking water at 5 mg Ba^{2+}/L and fed to rats over their lifespan had little or no effect on growth, carcinogenesis, or longevity.[125] Rats drinking water with 100 mg Ba^{2+}/L as $BaCl_2$ for 16 months exhibited significant but varying increases in systolic blood pressure.[126] In a similar study, the average systolic pressure increased significantly after exposure to 100 mg Ba^{2+}/L for one month and after 10 mg Ba^{2+}/L for eight months. After 16 months, rats

exposed to 100 mg Ba^{2+}/L had depressed heart rates and decreased cardiac function.[127,128] Another experiment examined the effect of $BaCl_2$ in drinking water for 92 days on serum electrolytes, body weight, behavior, and fertility in rats and mice.[129] The no observed adverse effect level (NOAEL) for Ba, based upon depressed body weight gain and renal and lymphoid lesions, was estimated to be 1,120 mg Ba^{2+}/L. Mortality was attributed to kidney damage. There appeared to be no adverse effects on reproduction and fertility, although there was a marginal reduction in pup weights. Tardiff et al.[130] studied acute oral and subchronic toxicity of Ba as $BaCl_2$ in rats. The acute oral LD_{50} for weanling rats was 220 mg Ba/kg BW and for adults was 132 mg Ba/kg BW. Drinking 250 mg Ba^{2+}/L for up to 13 weeks resulted in less water consumption than controls, but it did not cause any clinical signs of toxicity, nor was body weight affected. McCauley et al.[131] found no significant lesions in rats exposed to up to 250 mg Ba/L drinking water for 68 weeks, nor were there measurable electrocardiographic changes when measured at five months; however, Ba-exposed rats were more sensitive to norepinephrine.

An anecdotal report of $BaCl_2$ ingestion in a man suggests Ba may damage kidneys, although the kidney lesions were likely the result of IV therapy with $MgSO_4$.[120] Chronic Ba exposure causes nephropathy in rodents. Male rats drinking 5 ppm Ba as the acetate salt in a life-time study developed proteinuria.[125] McCauley et al.[131] identified kidney lesions in rodents administered 1,000 mg Ba/L in drinking water for 16 weeks. Dietz et al. identified kidney lesions in male and female mice receiving doses of 436-562 mg Ba^{2+}/kg BW/day via drinking water for 92 days. Rats receiving the same water drank less, receiving only about one quarter of the dose and had much milder renal lesions.[129] In a subsequent two-year cancer bioassay, female and male rats receiving 75 and 60 mg Ba^{2+}/kg BW/day, respectively, in drinking water gained less than controls but did not exhibit any Ba-related clinical signs. Mice in the same two-year study drank up to 160 (male) or 200 (female) mg Ba^{2+}/kg BW/day and had significantly greater premature mortality due to kidney disease than did controls.[132]

Most authorities indicate $BaSO_4$ is the least soluble and therefore least toxic form of Ba. A United Kingdom risk assessment of $BaSO_4$ described it as a low risk due to, among other reasons, low solubility.[133] Studies by Maglinte et al.[134] and several decades use of $BaSO_4$ as radiographic contrast media support the premise $BaSO_4$ is relatively nontoxic in mammals.[134-137] Ba sulfate-derived Ba was poorly bioavailable in an environmental study and thus did not bioconcentrate between trophic levels in the food-chain.[138] One exception is a study suggesting ^{131}Ba from $BaSO_4$ was nearly as bioavailable as $BaCl_2$.[139]

There are a number of reports regarding the toxicity of inhaled Ba.[140-143] Since the relationship between the toxicity of inhaled Ba and soluble Ba in feed or water is not fully understood, these studies were not included.

Summary

The acutely toxic effects of Ba are similar in monogastrics and ruminants. This argues that 1) Ba is a valid water quality concern for livestock and wildlife, and 2) subacute and chronic effects are probably similar, if not identical, between these species. The putative toxic mechanism(s) of the Ba^{2+} ion in rodents and human beings involve physiologic mechanisms that are highly conserved (i.e. very similar) throughout terrestrial mammals; therefore, any species-specific differences in toxicity logically derive from species-specific differences in the toxicokinetics of Ba. In monogastric mammals, the oral toxicity of Ba compounds correlates with their water solubility. Less soluble forms of Ba (notably $BaSO_4$) are poorly absorbed and are thus considerably less toxic than more soluble salts such as $BaCl_2$, barium nitrate ($Ba(NO_3)_2$), or barium hydroxide ($Ba(OH)_2$).[105] There is no equivalent data in ruminants. Theoretically, reduction of the SO_4 salt to sulfide by rumen microflora *might* result in increased bioavailability of the Ba^{2+} ion. There *is* some precedent for differences in metabolism of Ba between monogastrics and ruminants[144], but much more needs to be done. As a practical matter, however, this theoretical effect would be significant only in solid feedstuffs as insoluble forms of Ba (i.e. $BaSO_4$) will presumably not be present in drinking water in any significant concentration.

The long-term effects of Ba, especially on reproduction, have been incompletely investigated in any species. A single Russian report of Ba inhalation toxicity describes reproductive lesions in both male and female rats[145] whereas more recent rodent studies did not note alterations in reproductive tissues or reproductive function following acute-, intermediate- or chronic-duration exposure to Ba.[129,131,132] Kidney damage *was* observed in laboratory rodents following two-year exposure to 200 mg Ba/kg BW and in long-term (91-day) oral exposure to 450 mg Ba/kg BW, but it was not seen after administration of 250 mg Ba/kg BW for 36-46 weeks.[131,132]

The only *quantitative* data available in cattle indicates 138 mg Ba/kg BW, as $BaCO_3$, in dry feedstuffs was acutely toxic to steers, whereas 69 mg Ba/kg BW was not.[117] Assuming water consumption of 20% BW, this translates to 690 mg Ba^{2+}/L in drinking water as being acutely toxic or 345 mg Ba^{2+}/L as the NOAEL. This contrasts with the report of Richards et al.[116] that 2 mg soluble Ba^{2+}/L water, plus some undetermined amount of Ba from sediment, was immediately toxic to cows and calves. It is also much higher than the toxic dose reported in goats, where 7 mg/kg BW $BaCl_2$ (4.6 mg Ba/kg BW) was lethal.[115] It is likely that $BaCO_3$ in feed is not as bioavailable as the Ba^{2+} ion in water. The acutely lethal dose in the goat study translates to 23 mg Ba^{2+}/L under the assumptions outlined in the Introduction.

Obviously, much more research needs to be done with Ba in ruminants, but, given the current state of knowledge, *soluble Ba^{2+} concentrations should be held to well below 23 mg/L to avoid acute toxicity.* There is absolutely no data on *chronic* Ba^{2+} ion toxicity in any of our species of interest. This, plus the limited and conflicting data from chronic studies in other animals, makes it impossible to postulate a long-term "safe" level of the Ba^{2+} ion in drinking water for domestic livestock and/or wildlife species with any degree of certainty.

We do not recommend using water containing more than 10 mg Ba^{2+}/L even for short periods. Until there is better data, it is impossible to make any recommendations regarding chronic exposure.

4 Fluoride

Fluorine (F) is the most electronegative and reactive of known elements. It rarely occurs free in nature, chemically combining to form fluorides. Fluorides are widely distributed throughout the environment in various anthropogenic and natural forms. Mineral forms of F include cryolite (Na_3AlF_6), fluorite (CaF_2), and fluorapatite ($Ca_5(PO_4)_3F$). Vegetation can accumulate fluoride from soil, water, and the atmosphere.[146] In aqueous environments, F occurs as the free fluoride ion (F^-) and is mobile, especially in alkaline waters.[95] Unless surface waters are contaminated by a F^- source, ground waters tend to have higher concentrations of F^- than surface waters.

Essentiality

The NRC[147] describes F^- as an "important constituent" of bones and teeth, and, although essentiality has not been proven, small amounts have been added to municipal water supplies to improve dental health for decades. Apparently, even if F is essential, the dietary requirement is so small it is easily met by even highly purified diets. As noted by Ammerman,[148] "Whether or not it is essential for animals may be open to debate... The fact that it is toxic is more easily confirmed."

Metabolism

Fluoride is readily absorbed by the stomach, rumen, and small intestine. The efficiency of absorption depends upon the solubility of the specific F compound, other dietary components, and the species, sex, and age of the animal.[147,149] Conditions that result in very low pH favor the formation of hydrogen fluoride (HF), which is lipophilic and thus diffuses easily across lipid membranes. The F^- ion is absorbed in the small intestine via a pH independent process.[147] Soluble fluorides, i.e. the F^- ion in water, are almost 100% absorbed. Less soluble sources such as F compounds in bone meal are relatively poorly absorbed. Ca, Mg, Al, NaCl, and high lipid concentrations are known to depress F uptake.[147,150]

Two mechanisms are responsible for removal of F^- from the systemic circulation: renal excretion and deposition in calcified tissues. After absorption, most F^- circulates in plasma as ionic F^-. To a lesser extent, it circulates as CaF_2 or HF, or it is bound to protein.[150-152] Circulating F^- represents a relatively small portion of the total body burden but is the form most easily exchanged with other tissues and/or eliminated via renal filtration.[151,152] Urinary excretion is the primary route of elimination and is directly related to urinary pH; thus, factors that affect urinary pH influence how much F- is excreted.[150,153] Under "normal" circumstances, roughly 50% of ingested F^- is eliminated immediately, and the remainder is incorporated into bony tissues[147]; however, these percentages may be significantly modified by physiologic factors such as age, sex, or other factors. Calcified tissues such as teeth and bone have a great affinity for F^-, incorporating it as fluorapatite in place of hydroxyapatite in the calcified matrix.[147,153] To a certain extent, bone deposition represents a form of detoxication by decreasing the F^- exposure of other tissues. Fluorapatite crystals, however, are less soluble than the hydroxyapatite they replace and thus 1) persist for long periods in bone, and 2) interfere with normal turnover (remodelling) of bone. Therefore, at higher concentrations, bone F^- interferes with normal physiological processes like growth and healing. Since F^- deposition in skeletal tissues is related to the turnover of bone minerals, young, rapidly growing animals are more likely to accumulate it.[147]

Toxicity

Animals can ingest potentially toxic doses of F^- from a variety of sources. In the past, forages contaminated by aluminum smelters or grown in naturally high F^- soils, rock phosphate fed nutritional supplements, and/or consumption of naturally high F^- water have resulted in F^- poisoning.[147,149,154-156] Large doses of soluble F^- can form corrosive HF, interfere with ion gradients in excitable cells, and/or precipitate divalent cations from serum.[157,158] Thus, acute fluorosis is manifested as gastroenteritis, cardiac arrhythmias, and/or collapse.[157] Chronic or subchronic exposure to somewhat lower doses results in kidney damage,[157,159,160] neurologic damage, or reproductive failure.[161-163] The most sensitive (i.e. occur at the lowest dose) clinical manifestations of F^- toxicosis in livestock and wildlife under real-world conditions are tooth and bone deformities.[149,164-169] These bony tissue lesions often

result in difficulty grazing, reduced feed intake, ill-thrift, and decreased performance.[149,166,167] Alternating periods of high and low F^- exposure are more toxic than a continuous intake of the same average amount. Nutritional status and the age when exposed to F^- also influence tolerance.[170]

The effect of F^- on behavior and brain development was examined in rats by injecting pregnant dams with 0.13 mg/kg BW of sodium fluoride (NaF) subcutaneously on gestational days 14-18 or 17-19.[171] Weanlings received either no NaF or NaF in drinking water at 75, 100, or 125 mg F^-/L for six or 20 weeks, and three 3-month-old adults received water containing 100 mg F^-/L for six weeks. Rats exposed to F^- had sex-and dose-specific behavioral deficits. Males were most sensitive to prenatal exposure; females were more sensitive to weanling and adult exposure. Drinking water containing 125 mg F^-/L resulted in reduced growth, and 175 mg F/L was lethal. Shan et al.[172] treated rats with differing concentrations of F to investigate the effect of F^- on cognitive processes by examining its effects on nicotinic acetylcholine receptors (nAChRs) in the brain. Both 30 and 100 mg F^-/L in the drinking water produced subtle brain damage indicative of oxidative stress. Paul et al.[173] administered NaF by oral intubation daily at 20 or 40 mg/kg BW to adult female rats for 60 days and measured spontaneous motor activity, motor coordination, cholinesterase activity in blood and brain, and the protein content of muscle, liver, and serum. Sodium fluoride treatment suppressed spontaneous motor activity and tissue and serum protein concentrations in a dose-dependent manner. Wang et al.[174] found decreased total phospholipid concentrations and ubiquinone in rat livers due to oxidative stress after seven months of consuming water containing 30 or 100 mg F^-/L.

Rats were offered drinking water containing 225 mg F^-/L as NaF for 60 days.[175] A second group of rats was also gavaged with calcium carbonate ($CaCO_3$). The NaF-treated rats exhibited decreased food and water intake, reduced body weight gain, and impaired nervous function. Fluoride-induced dental lesions, inhibition of acetylcholinesterase and N^+K^+ ATPase activity, and decreased serum protein improved after NaF withdrawal. Calcium treatment lessened the impact of F by decreasing serum F concentrations.[175] Rats given drinking water with either 30 or 100 mg F^-/L as NaF for seven months had decreased kidney proteins and BW gains, and dental fluorosis.[159] Ten or 30 mg F/L administered to male rats via drinking water for three to six months did not cause any overt clinical effects but did produce biochemical indications of liver damage.[176] Oral administration of NaF in water at 5 or 10 mg (2.25 or 4.5 mg F^-)/kg BW/day for 30 days to adult male rats resulted in reduced body weight.[163] Testicular cholesterol and serum testosterone levels were not affected, but sperm motility and count were decreased resulting in a significant decline in fertility. Heindel et al.[177] studied fetal development in rats and rabbits fed up to 300 or 400 mg NaF (135 or 180 mg F^-)/L drinking water, respectively, during gestation. Although there were no teratogenic effects at any dose, dams lost weight after drinking water with concentrations greater than 150 mg NaF/L (rat) or 200 mg NaF/L (rabbit).

Certain elements are known to interfere with the uptake of F, a fact some have attempted to exploit therapeutically. Rats received drinking water treated with equivalent amounts of F as aluminum fluoride (AlF_3 0.5 mg/L) or NaF (2.1 mg/L) for 52 weeks to evaluate the interaction of Al with F.[178] AlF_3e reduced the neuronal density of the brain neocortex compared to the NaF and control rats. Brain and kidney Al concentrations were higher in both AlF_3- and NaF-treated groups than in controls. Rabbits were fed drinking water containing various combinations of either F^- (1-50 mg/L as NaF) or Al (100-500 mg/L as $AlCl_3$) for 10 weeks. Although none of the treatments resulted in significant weight loss, Al treatment decreased F^- accumulation in bone. Surprisingly, F, by itself, increased bone Al concentrations, suggesting Al or an Al-F complex play a role in osteofluorosis.[179] Kessibi et al.[180] gavaged sheep with 0, 1.9, or 4.7 mg F^-/kg BW, with or without 13.5 mg Al/kg, for 33 months. In all treated animals, the general health status declined and osteodental signs appeared while F^- levels increased in teeth, bones, and organs. In sheep given 4.7 mg F^-/kg BW, lesions were observed in kidney and liver. Aluminum sulfate ($(Al_2(SO_4)_3$) alleviated some, but not all, of the effects of 1.9 mg F^-/kg BW.

Rats and mice were fed no NaF in drinking water or at 11, 45, or 79 mg F^-/L for up to two years in a carcinogenesis bioassay.[181] Body weights and survival rates of F^- treated rats and mice were similar to controls. Osteosarcomas occurred in a small, statistically, and historically insignificant number of male rats at the highest dose, while there was no evidence of carcinogenic activity in female rats or mice of either sex. Rats on the highest two dosages also exhibited some increased osteo- and dental fluorosis.[181] Deer mice captured and fed 38, 1,065, 1,355, or 1,936 ppm dietary F^- for eight weeks

lost weight and many died at the highest dose.[182] The toxicological response and metabolism of F^- by three species of wild mammals (two species of voles and wood mouse) were compared to laboratory mice.[183] Animals were given no NaF or 40 or 80 mg F/L as NaF in their drinking water for up to 84 days. Forty and 80 mg F^-/L treatments caused mortalities in the voles, probably as a result of the greater water intake of this species. Severe dental lesions were apparent in all animals surviving the 80 mg F^-/L treatment.

Osteo-dental fluorosis was observed in cattle, buffalo, sheep, and goats from several villages in India where the mean F^- concentration in drinking water ranged between 1.5 to 4.0 mg/L.[164] Forage F^- concentrations were not measured, but the situation described suggests only background concentrations were present. The prevalence and severity of skeletal fluorosis increased with increasing F^- concentration and age. Cattle and buffalo near a phosphate plant in India developed dental and bony lesions due to fluorosis.[184] Lesions were more common in older animals than younger and in buffalo than in cattle. Environmental F^- concentrations were: 534 mg F^-/kg in fodder, 1.2 mg F^-/L in pond water, and 0.5 mg F^-/L in ground water, which, assuming standard consumption of forage and water, would have provided approximately 14 mg F^-/kg BW. Two ranches, one with drinking water containing up to 10.5 mg F^-/L, the other with 3 mg/L, were compared in Argentina.[185] While both had similar forage F^- values (~15-25 ppm), cattle from the former exhibited excessive dental erosion. Neeley and Harbaugh[186] studied a Texas dairy herd drinking 4-5 mg F/L before and after management changes that resulted in increased F^- intake from 0.52 mg/kg BW to 1.69 mg/kg BW, primarily as a result of increased water consumption. Most animals exhibited dental fluorosis both before and after the change, but breeding efficiency and production increased after the change, probably as the result of better nutrition and management. Rand and Schmidt[187] followed several Arizona herds' drinking water containing 16 mg F^-/L and consuming forage containing up to 25 ppm F^-. They concluded 1 mg F/kg BW can be tolerated for 5-10 years with only minor cosmetic effects, but 2 mg F^-/kg BW will result in accelerated tooth wear and signs of osteofluorosis. After the Lonquimay volcano erupted in Chile, animals were exposed to water concentrations less than 2 mg F^-/L and as much as 48 ppm F^- in forage. Two years later cattle were still developing fluorosis.[188] Cattle fed a contaminated supplement that provided between 0.7-1.6 mg F^-/kg BW/day for a year developed bone lesions and dental fluorosis.[189]

Merriman and Hobbs[190] conducted an extensive five-year study of the interactions between soil and water F^- and nutrition in cattle. Cattle on pastures with average forage concentrations of 143 ppm F developed dental fluorosis. Fluoride in soil, water, and grasses reportedly did not affect gain, but the experimental design was too small to reliably detect differences in performance. Suttie et al.[191] fed dairy calves 1.5 or 3 mg F^-/kg BW, either continuously or in a six-month rotation, for six years. Bone F^- was related to total intake and urinary F^- remained high during the "off" period, but dental lesions were related to the F^- concentration when teeth were being formed. None of the treatments "affected growth or reproduction," but only a small number of animals was studied. The physiologic effects of forage contaminated by fumes from an aluminum smelter and NaF-treated forage were examined in cattle.[192] Gains were significantly less on diets containing more than 200 ppm F^- as NaF. Cattle receiving 70-100 ppm F^- exhibited decreased reproductive efficiency indirectly attributed to dental fluorosis.

Cattle were fed 134 ppm dietary F^- as soft phosphate or CaF_2, or 67 ppm F^- as NaF for 91 days to compare the bioavailability and toxicity of the different sources of F. Feed consumption, average daily gains, and feed conversion were not influenced by source of F[193]; however, the study was too short to detect dental effects, and no controls were included. Shupe et al.[194] summarized 30 years of experimental and observational studies in cattle, sheep, horses, deer, elk, and bison exposed to differing F^- concentrations. They concluded that excessive dietary F^- during tooth formation damages teeth, and the abnormal wear of these teeth results in impaired performance. Offspring from F^--damaged animals showed no signs of fluorosis. Short-term exposure of dairy heifers to 2.5 mg F^-/kg BW during 13-15 or 16-18 months of age resulted in severe dental fluorosis, even though the *total* F intake was not excessive.[195] Eckerlin et al.[196] and Maylin et al.[197] described a farm where F^--contaminated concentrate contributed approximately 12.8 to 56 ppm F^- to the diet. Cows had depressed milk production, while their calves exhibited dental and osterofluorosis and had severely stunted growth.

Holsteins were raised and maintained for 7.5 years on forage containing 12, 27, 49, or 93 ppm F^- as NaF.[198] Initially, F had no effect on feed intake, digestion coefficients, or nutrient absorption. However, after the cows had been through two lactations, cattle consuming the higher two F^- diets consumed and digested less. The "tolerance level" for dietary F was concluded to be between

27-49 ppm of the total diet. McLaren and Merriman[199] monitored beef cattle maintained on "high" (125 ppm) or "low" (37 ppm) F pastures and "good" (80-100% of NRC recommendations) or "poor" nutrition in a 2x2 factorial experiment. They concluded neither F concentrations nor plane of nutrition had any effect on the "general health" of the animals. Sixteen dairy heifers were fed hay containing 10 or 62 ppm F^- or 10 ppm hay supplemented with CaF_2 at 69 ppm F^- or NaF at 68 ppm F^-, for 588 days.[200] The F diets resulted in dosages of 1.14, 1.32, or 1.29 mg F^-/kg, respectively. Calcium fluoride was less toxic than contaminated hay or NaF. Fluoride caused no adverse effects on soft tissues but did cause significant damage to teeth and bones. Van Rensburg and de Vos[201] fed cows 5-12 mg F^-/L in drinking water, with or without superphosphate, through four breeding seasons. By the second season the 8 and 12 mg F^-/L groups exhibited prolonged post-parturition anestrus, and fertility declined in those two groups during the third season. By the fourth season toxic effects were apparent in the 5 mg F/L group as well.

Sheep were maintained on 2, 5, or 10 mg F/L drinking water from natural sources for four years.[202] The highest dose affected wool production, probably as a result of limited food consumption caused by dental fluorosis. Pregnant ewes drinking 10 mg F/L water did not transmit toxic quantities of F^- to the fetus or to the lamb through milk. Sheep fed a commercial, non-defluorinated, rock phosphate lick to provide 2 mg F/kg BW showed signs of fluorosis.[203] Thirty-seven % of the commercial flock was affected, as opposed to only 17% of the breeding animals of the same age that were in better nutritional condition. Cattle and sheep raised on South African farms where water F^- ranged from 4-26 mg/L and forage from 5.3-22.4 ppm experienced severe osteo- and dental fluorosis.[204] Assuming normal consumption of forage and water, the dose received was between 2-4 mg F^-/kg BW. A ranch in New Mexico experiencing dental fluorosis was discovered to have water sources varying from 0.09-3.32 mg F^-/L. Interestingly, the higher concentrations occurred in wells only used during part of the year, and a child drinking from the highest well had dental fluorosis as well.[205]

Horses and swine are believed to be less susceptible to fluorosis than cattle and sheep, but there is little clinical and no experimental data to support the contention.[147] Horses grazed in F^- contaminated areas developed similar fluorotic signs as cattle and sheep.[206] Swine received no NaF or were fed 200 or 1,000 ppm dietary F^- as NaF for 45 days in an experiment to determine the effects of F^- on bone growth.[207] The higher concentrations of F^- produced dose-related decreases in bone growth, feed consumption, and overall growth. Bones from F^--treated pigs were less dense and exhibited growth plate abnormalities.

Fluoride toxicosis was diagnosed in elk, deer, and bison on the basis of lesions and F^- concentrations in teeth and bones collected by hunters in Utah, Wyoming, and Idaho. Vegetation and water samples collected from areas where fluorotic animals were discovered contained 5.5-430 ppm F^- and 0.5-24 mg F^-/L, respectively.[208] The investigators concluded that geographic areas where domestic livestock have chronic fluorosis are also problematic areas for wildlife, which implies wildlife are roughly equally sensitive to F. Suttie et al.[209] studied deer near a new aluminum smelter where forage concentrations ranged from 1-30 ppm F^-. All deer had increased dental F^- and some mild cosmetic fluorosis, but none exhibited excessive tooth wear. Tissue F concentrations in deer diagnosed with fluorosis on the basis of dental lesions near an aluminum smelter were similar to those reported for cattle.[210] Suttie et al. fed 25 or 50 ppm F^- as NaF to whitetail deer fawns.[211] Fluorotic lesions were similar to those in cattle fed the same amount, but cattle seem to develop more bony lesions and fewer dental lesions than deer. Vikoren et al.[212] surveyed several hundred jawbones from moose, red deer, and roe deer near aluminum smelters where forage concentrations averaged 30 ppm F^-, and they also looked at a small number of sheep in the same area. Although the overall incidence of fluorosis was low, tissue concentrations were similar in sheep and cervids, and the threshold bone F^- concentration for disease in cervids was similar to what was previously reported in domestic species.

Summary

Fluoride generates considerable controversy in human health, largely as a result of the common practice of fluoridating municipal water supplies. We were not, however, able to find any convincing experimental studies that suggested the dramatic effects associated with acute exposure to relatively large doses of F^- carried over to low level, long-term exposure. Although there are a few reports of dental fluorosis in people at slightly lower concentrations, the current primary drinking water standard for human consumption is 4 mg F^-/L; the secondary standard, apparently based upon cosmetic dental effects, is 2 mg F^-/L.

Our search of the literature pertaining to fluorosis in animals yielded similar results. We were not able to find *any* reports of toxic effects in livestock or wildlife that occurred at lower F^- dosages than cause osteo-dental fluorosis. Thus, as a practical matter, maximum tolerable concentrations of F^- in water for livestock and wildlife should be based upon dental and osteal effects.

The effects of F in feedstuffs and water are additive; what really counts is the *total* dose of biologically available F^- ingested by the animal. Most of the reports we reviewed, when reduced to mg F^-/kg BW, indicate the threshold dose for chronic osteo-dental fluorosis in cattle is approximately 1 mg F^-/kg BW. This is in agreement with the NRC,[147] which indicates 30-40 ppm dietary F^- (which translates to 0.75-1.0 mg F^-/kg BW) is the tolerance level for the more sensitive (i.e. during dental development) classes of cattle.

Numerous studies have demonstrated the susceptibility of wild ungulates (deer, elk, etc.) to fluorosis; however, there has been only one controlled experiment[211] from which dose-response can be extrapolated. A few other epidemiologic studies provide sufficient data to form rough estimates of the amount of F^- required to produce signs of fluorosis in cervids. Taken together, these indicate wild cervids are approximately as sensitive to the toxic effects of F^- as cattle. Sheep seem to be slightly less sensitive than cattle although one Australian report indicated long-term exposure to as little as 10 mg F^-/L drinking water in Queensland decreased wool production.[202] Given temperatures in that region, water consumption probably resulted in 1-2 mg F^-/kg BW for much of the year. The sole report that included any data on horses suggested horses are two-fold more tolerant than cattle, but it goes on to describe situations in which ruminants and horses, sharing a pasture and water supply, were similarly affected.

Assuming Wyoming forages normally contain less than 10 ppm F^-[213], a water concentration of 3.75 mg F^-/L would be required to achieve the 1 mg F/kg BW necessary to cause fluorosis in cattle, and water containing less should not cause measurable production problems.

We recommend water for cattle contain less than 2.0 mg/L F^-. By extension, these waters should also be safe for sheep, cervids, and probably horses.

5 Molybdenum

Molybdenum (Mo), an essential trace element required for nitrogen fixation and the reduction of nitrate to nitrite in plants and bacteria, is widely distributed in nature.[214] Geochemical surveys in England found that Mo content in soil and sediment corresponds closely to underlying black shales.[215] Other sources of Mo in the environment include industrial contamination by metal alloy manufacturing, copper mining, and coal mining.[216-221] Molybdenum occurs predominately as the molybdate (MoO_4^{2-}) ion in natural water sources, and concentrations are typically very low (<2-3 µg/L), unless contaminated by an outside source, in which case they can reach 25 mg Mo/L.[222,223] In forage, Mo concentrations vary and depend on the Mo concentration, moisture content, and the pH of the soil.[222,224] Alkaline environments greatly increase the bioavailability of Mo to plants, and thus increase the likelihood of Mo toxicity in grazing animals.[222,225] Surveys have identified extensive areas of forage containing potentially toxic concentrations of Mo (10-20 ppm) in at least five western states, including Wyoming.[226]

Essentiality

Mo is an essential element for mammals due to its involvement in the enzymes aldehyde oxidase, sulfite oxidase, and xanthine oxidase.[214,227] As a cofactor for these enzymes, it aids in catalyzing the oxidation or metabolism of sulfur-containing amino acids, purines, pyrimidines, and aldehydes.[214] Experimentally, dietary Mo deficiency decreased feed consumption and caused a 25% reduction in live-weight gains in adult goats, and kids from deficient dams gained less compared to control animals.[228,229] Reproductive effects of deficiency include decreased pregnancy rates and higher mortality in offspring.[227-229] Dietary requirements are so low (about 100 ppb DM), however, that deficiency is very rare under natural conditions.[227]

Metabolism

Once ingested, Mo is absorbed in the stomach and throughout the small intestine. In the small intestine, MoO_4 is actively transported across the mucosal epithelium via the same carrier-mediated transport mechanism that transports sulfate (SO_4).[230] The administration of SO_4 to *monogastric* animals consuming a Mo-rich diet decreases blood Mo concentration and increases excretion, potentially alleviating toxicity.[230-234] Interestingly, dietary S increases the toxicity of Mo in ruminants, presumably as a result of thiomolybdate formation in the rumen. Molybdenum is transported in the blood as MoO_4, where it is distributed to tissues for integration into enzyme systems. Excretion is primarily via urine; however, feces and milk can also serve as important routes of removal.[214,235-238] The rate of absorption differs amongst species, age groups, and sex. For example, after orally administering Mo to swine and cattle, it was found that Mo peaked in the blood of swine after two to four hours, while it took 96 hours to peak in cattle.[236] Water soluble forms of Mo, such as ammonium molybdate ($(NH_4)_6Mo_7O_{24}$), sodium molybdate (Na_2MoO_4), and Mo from forage, are more readily absorbed than their organic counterparts.[214]

The ruminal interaction between Mo, SO_4, and Cu is responsible for the greater sensitivity of ruminants than monogastrics to Mo. In the rumen, sulfur compounds (mostly SO_4) are reduced to sulfide by rumen microbes. Sulfide then combines with MoO_4 to form either tri- or tetrathiomolybdates (TM).[214,239,240] Thus, in ruminants, relatively little MoO_4 or S reach the small intestine to be absorbed as such. In the rumen, TMs bind irreversibly to the solid phase of digesta and act as powerful chelators of Cu, retaining it in the gut. As a result, Cu absorption from the GI tract is decreased as much as 88%.[241-245] Trithiomolybdates can also enter the circulation[241,246] where they bind cooperatively with Cu to albumin, resulting in decreased availability of Cu for critical functions. Because this complex is insoluble in trichloroacetic acid (TCA), the term "TCA-soluble" Cu is often used as a synonym for biologically useful Cu.[241,245,247-249] As a consequence of the binding to albumin, biliary excretion of Cu is enhanced, less Cu is incorporated into enzymes such as ceruloplasmin (Cp), and less Cu is stored in tissues. The decreased availability of Cu for enzyme synthesis impacts a number of physiologic processes, including immune function and bone and elastin formation.[242,245,250-261] Trithiomolybdates bound to albumin are

relatively stable; however, once unbound, TMs are rapidly hydrolyzed to MoO_4 and SO_4.[246,249,262]

Since the clinical effects of molybdenosis are in large part due to secondary Cu deficiency, it is useful to review Cu metabolism. Although Cu is an essential trace element for all mammals and deficiency is frequently associated with a number of maladies, the Cu ion itself is quite toxic to cells. Thus, the metabolism of Cu in mammals involves a number of different carrier and storage proteins that bind Cu, permitting it to be absorbed, distributed, and eliminated without exposing cells to excessive amounts of the free ion. Copper is absorbed in the intestine by carrier proteins and stored in mucosal cells as a protein complex.[263] In monogastric species such as rats, horses, and swine, most uptake occurs in the small intestine. In ruminants, there is some evidence significant absorption also occurs in the large intestine.[245] Copper is transported to the liver bound to albumin and transcuprein, where the proteins and their bound Cu atoms are taken up into hepatocytes.[263] Within the liver cells, Cu is distributed between various storage proteins, especially metallothionein (MT), microsomes, nuclei, lysosomes, and the cytosol.[245,263] Copper is exported from the liver to the rest of the body for incorporation into enzymes as a protein complex with Cp.

Toxicity

Despite the fact Mo is intrinsically (i.e. without metabolism to TM) toxic[245,264,265], secondary Cu deficiency is the most common pathogenesis underlying molybdenosis. The form of Mo ingested and, more importantly, the Cu:Mo ratio are critical determinants of toxicity.[266-268] Cu:Mo ratios of 2:1 or less result in clinical signs, and effects are exacerbated by high dietary S.[267] Various authorities have recommended Cu:Mo ratios of 4:1 or greater as the minimum "safe" ratio.[214,267,269] Signs of *acute* Mo toxicity include gastrointestinal irritation, diarrhea, liver and kidney damage, and, ultimately, death.[253,267,270] Diarrhea appears to be a direct effect of Mo on the intestinal mucosal cells, rather than Cu deficiency.[245] In chronic poisoning, anorexia and weight loss are initial clinical signs, followed by diarrhea, anemia, depigmentation of the hair coat (achromotrichia), ataxia, and bone and joint deformities.[214,267,271,272] Integumentary lesions and bone and joint deformities are probably due to deficiencies of several Cu-dependent, critical enzymes, as well as possibly decreased P in bone.[267,273,274] Decreased reproductive function, including decreased libido and fertility, has also been associated with molybdenosis.[266,275-278]

Acute to subacute toxicity has been demonstrated experimentally and occurs naturally in cattle, buffalo, and mule deer. The accidental addition of Na_2MoO_4 at the rate of 19,000 ppm (estimated Mo concentration 7,400 ppm) to cattle rations resulted in decreased feed intake, hind limb ataxia, profuse salivation and ocular discharge, diarrhea, liver and kidney damage, rough hair coat, and death.[270] Feeding 1.36 g Mo per head per day to five cows for an unspecified amount of time produced extreme scouring and loss of condition in three animals.[279] After consuming a ration containing 10.5 ppm Cu and 140 ppm Mo for three to four days, Holstein-Friesian lactating cows and steers developed hemorrhagic diarrhea and front limb lameness, and they died.[280] Contaminated grazing pastures with forage Mo concentrations between 16-24 ppm and 6-11 ppm Cu resulted in acute diarrhea, loss of condition, and posterior stiffness in cattle.[221] Feeding 2,000 ppm Mo as $(NH_4)_6Mo_7O_{24}$ for three days resulted in diarrhea and feed refusal in cattle.[281] After grazing a pasture contaminated with used motor oil containing molybdenum bisulfide for two weeks, cattle exhibited diarrhea, anemia, decreased milk production, achromotrichia, and hind limb weakness.[282] Four male buffalo were given 5 mg Na_2MoO_4 (2.35 mg Mo)/kg BW/day for 180 days; by two weeks, clinical signs included diarrhea, decreased weight gains, incoordination, swelling of hind fetlocks, and irregular hoof wear.[283] Including 2,500 ppm Mo as Na_2MoO_4 (equivalent to approximately 62.5 mg Mo/kg BW) or more in the diets of mule deer for 33 days resulted in diarrhea and feed refusal; whereas, smaller doses were without apparent effect. The authors concluded that deer are relatively resistant to Mo, compared to cattle.[284]

Relatively few studies of molybdenosis have been conducted in monogastrics. Most of these involve relatively large doses of Mo and don't seem to involve Mo-Cu-S interactions.[233,285-288] Rats given a diet containing 1,000 mg Na_2MoO_4/kg BW for five weeks gained significantly less than controls, and 50% developed mandibular exostoses.[289] Feeding rats 1,200 ppm Mo as Na_2MoO_4 for six weeks resulted in decreased weight gains, and skin, tail rings, and femurs all required much less force to break compared to control tissues.[290] Six hundred ppm Mo fed to rats for three weeks caused a depression in nutrient utilization and decrease in gain.[286] Rats consuming 800 ppm Mo for five weeks showed a 36% depression in growth; adding 0.29% SO_4 largely prevented this effect.[233] Male rats given 1,200 ppm Mo as Na_2MoO_4 for four to five weeks showed a 53% reduction in growth as

well as reduced feed intake, followed by death.[285] One thousand ppm dietary Na_2MoO_4 resulted in weight loss, diarrhea, hair changes, palpable mandibular nodules, and other bone and joint abnormalities in rats.[291] Rats fed 20 ppm Mo and 5 ppm Cu showed significant reduction in gains and depigmentation of hair.[275] Rabbits consuming 1,000 ppm Mo as Na_2MoO_4 in a diet with 16.4 ppm Cu developed anorexia, alopecia, slight dermatosis, and anemia, and they died after four weeks.[273] Feeding rabbits 4,000 ppm Na_2MoO_4 (roughly 55 mg Mo/kg BW) for four weeks resulted in anemia, abnormal bone development, and degeneration of myocardium and skeletal muscles.[292] Ponies fed up to 102 ppm Mo with normal (9 ppm) dietary Cu remained asymptomatic for 50 days.[293] Similar results were reported in colts fed 50 ppm dietary Mo.[287] Four horses fed 20 ppm dietary Mo as ^{99}Mo did not exhibit any signs of molybdenosis or deranged Cu metabolism. The radioactive label in plasma was bound to the MoO_4 ion rather than TM, as seen in ruminants.[288]

Chronic toxicity has been investigated both in field studies and experimentally in cattle, sheep, and ruminant wildlife. Forage containing 24-28 ppm Cu and 14-126 ppm Mo that had been contaminated by a metal alloy manufacturing plant resulted in emaciation and diarrhea in cattle beginning four weeks after introduction to the affected pasture.[219] Cattle grazing pasture contaminated by aluminum alloy plants at a concentration of 77.5 ppm Mo exhibited severe scouring, loss of condition, decreased milk production, and achromotrichia.[220] Cattle grazing pastures containing 1-20 ppm Mo exhibited diarrhea, listlessness, and abnormal hair color compared to animals grazing pastures containing < 1 ppm Mo with no clinical signs.[218] Feeding two male calves, with average body weights of 460 pounds, 4.0 g Na_2MoO_4/day resulted in diarrhea, discolored hair, weight loss, anemia, and decreased libido.[266] Cattle consuming a diet containing 4 ppm Cu and 5 ppm Mo exhibited decreased weight gains, decreased feed intake, and abnormal hair texture and color after 16-20 weeks.[264] Pastures containing 25.6 ppm Mo produced severe diarrhea, emaciation, anemia, faded hair color, salt craving, and death in grazing cattle.[294] Yearling steers given 1.5 mg Mo/kg BW for 150 days and grazed on pasture containing 0.32% S developed diarrhea, inflammation of the sheath, rough hair coat, and anemia.[295]

Grazing forage containing 4-14 ppm Cu and 95-460 ppm Mo and drinking water with 14 µg Cu/L and 7,200 µg Mo/L for 11 weeks resulted in watery diarrhea, rough hair coats, and a stiff shuffling gait in 50% of cow-calf pairs.[296] Forage contaminated with 2-220 ppm Mo caused diarrhea, roughening and discoloration of the hair coat, and weight loss in grazing cattle.[297] Forage concentrations of 16.5-23.5 ppm Mo and 2-25 ppm Cu resulted in diarrhea, weight loss, and achromotrichia in cattle.[298] Feeding 100 ppm Mo to heifers for 11 months resulted in anemia, scouring, achromotrichia, and weight loss. Five of 16 died two weeks after termination of the experiment.[299] Heifers receiving diets containing 100 ppm Mo and 0.3% S as SO_4 became emaciated and diarrheic after one month at this treatment level. Heifers given 5-20 ppm Mo with 0.3% S or 50 ppm Mo without added S did not exhibit any signs of illness, but they did have decreased tissue Cu compared to controls.[300] Weight loss and scouring were evident in cows ingesting 173 ppm Mo for two months. Diets containing 53 and 100 ppm Mo did not produce clinical signs but did interfere with Cu metabolism.[301] Forage containing 6-36 ppm Mo resulted in emaciation in cattle with severe diarrhea, anemia, achromotrichia, and swollen genitals.[302] Water containing 50 ppm Mo induced signs of secondary Cu deficiency in five-week-old calves.[303] Moose in Sweden have been reported to exhibit signs of molybdenosis similar to cattle, including diarrhea, emaciation, achromotrichia, sudden heart failure, and osteoporosis; however, no levels of Mo in the forage or water sources were reported.[304]

Sheep grazing pastures containing 5.5-33.5 ppm Mo and 6.0-8.7 ppm Cu for 76 weeks developed hemorrhaging around the femoral heads, tuber sacrales, and psoas muscles. Exostoses were frequent on humeri and femurs, and the periosteum appeared to have lifted from the bone surface.[225] Feeding ewes 1 kg commercial grass cubes with 5 ppm Cu and supplemented with Na_2SO_4 and $(NH_4)_6Mo_7O_{24}$ to provide 10 g SO_4 and 50 mg Mo, respectively, until one month prior to lambing resulted in incoordination of front and hind limbs and a marked ataxia in lambs within 60 days of birth.[305] Sheep grazing pastures with 20 ppm Mo and 5-7 ppm Cu developed connective tissue lesions including lifting of the periosteum and hemorrhaging in periosteum and muscle insertions.[306] Drenching (i.e. dosing by stomach tube) goats daily with $(NH_4)_6Mo_7O_{24}$ to provide 50 ppm dietary Mo for 235 days caused general debility, depigmentation of hair coat, and weight loss.[307]

Notwithstanding other work, several studies have failed to produce any adverse health effects after Mo ingestion. Grazing cattle on pastures containing 13 ppm Mo and

supplementing with 17 ppm Cu for six months resulted in no signs of adverse effects.[308] Cattle grazing a reclaimed mine tailing site containing 21-44 ppm Mo and 13-19 ppm Cu for three consecutive summers exhibited no signs of Mo toxicity.[217,309,310] No adverse effects were noticed in a cannulated steer consuming sun-cured hay containing 49.68 ppm Mo and 19.09 ppm Cu.[311] In each of these studies, low S in both the diet and water was offered as being a possible explanation for the lack of clinical signs observed.

Summary

Horses, like most monogastric animals, are very resistant to the effects of Mo as compared to ruminants. Cattle are commonly cited as slightly more susceptible to molybdenosis than sheep, and limited quantitative data suggest mule deer are relatively resistant compared to cattle. Therefore, drinking water Mo concentrations that are safe for cattle are probably also safe for horses, other classes of livestock, and wild cervids. Although there is quite a bit of variability in the reports summarized above, and some (large) amount of dietary Mo may cause poisoning regardless of Cu status, the bottom line seems to be that total dietary Cu:Mo ratios of less than 2-4 can result in chronic toxicity and decreased production in cattle, especially if dietary S is higher than absolutely necessary.

As with many substances, the effects of forage and water Mo concentrations are additive, and, in some areas of the western United States, forage Mo concentrations are already toxic or very nearly toxic. Under these conditions, *any* additional Mo intake contributed by drinking water is potentially dangerous. In these areas, however, producers are likely already aware of the problem and feeding supplemental Cu. A more normal situation would be cattle grazing "typical" Wyoming forage containing 7 ppm Cu (or supplemented to that level) and negligible Mo (~1 ppm). Under these conditions the critical safe 4:1 ratio would be exceeded whenever drinking water contains 375 µg Mo/L.

We recommend that, in the absence of other data, drinking water for livestock and wildlife contain less than 0.3 mg/L. **If dietary Mo is higher, which is not unusual in this region, water Mo concentrations should be adjusted downward accordingly.**

6 Nitrate and Nitrite

The nitrate (NO_3^-) and nitrite (NO_2^-) ions are intermediates in the biological nitrification cycle and the primary source of nitrogen (N) for plants in the soil. Plants accumulate NO_3^- from soil to synthesize protein via a multistep process; excessive NO_3^- accumulation as a result of this process may cause poisoning of grazing animals.[312] Nitrate or NO_2^- may contaminate water as a result of contact with natural minerals (e.g. niter), agricultural runoff (fertilizer, manure) or industrial processes.[312-314] Nitrate is the more stable of the two N species and therefore more common in surface waters.[315] Nitrite (NO_2^-) usually results from biological reduction of NO_3^-, but it may also be an industrial contaminant or exist in ground waters where pH and redox potential prevent oxidation to NO_3^-.[315] Both ions are extremely water-soluble and therefore water-mobile.

Essentiality

Although N is an essential macro element for mammals, NO_2 and NO_3 are not essential *per se.*

Metabolism

While the NO_3^- ion is readily absorbed in the upper gastrointestinal (GI) tract and possesses intrinsic toxicologic properties such as vasodilation, the condition referred to as "nitrate poisoning" actually depends upon reduction of NO_3^- to NO_2^- in the upper GI tract.[312,316-318] This process occurs in human infants[319,320], and it is the basis of current human drinking water standards, but nitrate toxicity is primarily a problem in ruminant species, as the rumen microflora are well-suited to catalyze NO_3^- reduction. Radiotracer studies indicate that NO_2^-, formed from NO_3^- in the rumen, may either be further reduced and incorporated via NH_3 into amino acids or reduced via nitric oxide to N and expelled.[321] Unfortunately, neither of the latter pathways (i.e. amino acids or N) is as fast as the initial reduction to NO_2^-, and dangerous NO_2^- concentrations accumulate in the GI tract when assimilatory pathways are overloaded.[321-323] Under the proper dietary conditions (mainly adequate carbohydrates) the assimilatory pathways can adapt to high NO_3^- concentrations, and very little NO_3^- or NO_2^- escape the rumen.[314,322,324-327] Sustained exposure to moderately high NO_3 diets results in induction of the assimilatory pathways, and this ability may be acquired by transfer of GI flora from one ruminant to another.[322,323] Once absorbed into the bloodstream, the NO_3^- ion is rapidly distributed throughout the body water and excreted via urine and saliva, whereas the NO_2^- ion is oxidized to NO_3^- via a coupled reaction with hemoglobin and eliminated as NO_3^-.[316,318,328,329]

Other differences between ruminants and monogastrics in the metabolism of the NO_2^- and NO_3^- ions, while not directly tied to the ruminal metabolism of NO_3^-, probably reflect evolutionary pressure of the constant background of NO_2^- ruminants receive from ruminal metabolism. The blood $t_{1/2}$ of NO_2^- is similar in monogastrics and ruminants, but the elimination of NO_3^- from blood is much slower in monogastrics[316] and the normal background metHb concentration is higher in monogastrics.[330] In ruminants only a small portion of an oral dose of NO_3 is eliminated in the urine, whereas in monogastrics most ingested NO_3 is eliminated via urine.[314]

Some sources suggest the NO_3^- ion is more bioavailable in water than feedstuffs.[312-314] The experiments this conclusion was drawn from, however, were based upon aqueous NO_3^- administered directly into the stomach vs. contaminated feedstuffs offered ad libitum.[314] The most important determinant of NO_3 toxicity seems to be how rapidly a given dose of NO_3^- is administered, rather than whether it is in feed or water.[314,331,332] For example, the single oral median lethal dose ($SOLD_{50}$) of $NaNO_3$ in cows was reported to be 328 mg/kg BW when given at once, but when the same dose was spread over 24 hours, the LD_{50} increased to 707-991 mg/kg.[331]

Toxicity

Although the most common source of NO_3 poisoning in livestock is contaminated feedstuffs, NO_3^--contaminated drinking water *has* poisoned people and animals.[320,333-336] The mechanism of poisoning involves GI microbial reduction of the NO_3^- ion to NO_2^-, which is absorbed into the bloodstream where it oxidizes the ferrous iron atoms (Fe^{+2}) in hemoglobin (Hb) to the ferric (Fe^{+3}) state, resulting in methemoglobin (metHb), which can-

not transport oxygen from the lungs to the rest of the body. The exact mechanism by which Hb is oxidized is the subject of some controversy but is currently thought to be a multi-step autocatalytic process involving several free radicals.[328] The end product of this reaction is NO_3^-, which is eliminated via urine and saliva. As might be expected, the clinical signs of acute NO_3 poisoning (cyanosis, hyperpnea, muscle tremor, weakness, collapse, and death) reflect the effects of anoxia on critical organs such as the brain and heart. Pregnant animals that survive episodes of acute NO_3 poisoning during the latter part of pregnancy may abort within one to two weeks.[333,337-340] Poisoning during earlier pregnancy does not usually result in abortion.[313,331,333,341]

As might be expected from the toxic mechanism, monogastric animals (with the exception of human infants) seem to be relatively resistant to the effects of NO_3 compared to ruminants. Textbooks suggest NO_3 is approximately 10-fold more toxic in ruminants than monogastric animals.[317] Burwash et al.[342] fed six mares high NO_3 (1.7-1.85%) oat hay for 13 days. Although the serum NO_3^- concentration increased significantly, there was no change in metHb concentration, no effects on blood chemistry parameters, and no clinical signs of poisoning. They concluded it is safe to feed horses diets containing 2% NO_3 and "likely much higher" concentrations. Mice exposed to drinking water containing 1,000 mg NO_3^-/L for 18 months excreted more ammonium ion than controls early in the study and may have died slightly sooner (17.5 months vs. 18 months) but did not show any signs of poisoning.[343] Seerley et al.[344] fed water containing 1,465, 2,900, or 4,400 mg NO_3^-/L as $NaNO_3$ to weanling pigs for 84 days with no effect upon rate of gain, water consumption, or clinical signs of toxicity. The difference in toxicity between animal species is not nearly as pronounced for NO_2.[317] Although rare, monogastric animals have been acutely poisoned by the NO_2^- ion from water[335] and feedstuffs.[345]

Wright and Davison[346] reviewed the literature and concluded the LD_{50} of NO_3 in ruminants was between 700-985 mg NO_3/kg BW when fed as dry feed. Experimentally, sheep have been acutely poisoned by NO_3 doses as low as 300 mg/kg BW[323,325,329,347]; however, doses as large as 800 mg/kg BW have been fed without measurable effects.[323,326,327,340,347,348] Field reports have incriminated feedstuffs containing as little as 2% ppm dietary NO_3 (which would provide about 500 mg NO_3/kg BW) as causing acute lethality in sheep .[349,350] Experimentally, 3.4% dietary NO_3, fed as pigweed or oat hay killed two of five ewes. The lethal dosages, calculated from consumption data, were 660 and 730 mg NO_3/kg BW, respectively. Campagnolo et al.[351] reported the accidental poisoning of several animals, including sheep, by water containing 6,000 mg NO_3^-/L at a county fair; however, the water contained several other substances that might also have been toxic.

Cattle have been experimentally poisoned by 520 mg/kg BW or more NO_3 incorporated into feedstuffs[341,346,352,353], and as little as 200 mg/kg BW may be toxic if given by gavage.[354] Several investigators[324,354,355] consistently produced sublethal toxicity with 200-300 mg NO_3/kg BW in order to test various protective strategies. Other investigators[322,356,357] failed to demonstrate toxicity at dietary concentrations as high as 0.9% (approximately 225 mg NO_3/kg BW), although one[322] reported that sustained exposure enhanced the ability of the rumen microflora to degrade NO_3^- and NO_2^-. Calves were experimentally poisoned by drinking water containing 2,500 mg NO_3^-/L (250 mg/kg BW), but none were affected by 2,000 or less.[338] Older texts and reviews variously describe the minimum toxic dose in cattle as 169-500 mg NO_3/kg BW.[313,317,358]

There are numerous anecdotal reports of acute NO_3 poisoning in cattle associated with contaminated feedstuffs. O'Hara and Fraser[359] summarized 10 episodes of acute NO_3 poisoning in New Zealand in which mortality varied from less than 1% to almost 50%. Forage concentrations associated with these cases ranged from 0.3-5.3% NO_3 (mean = 3.3%) with variations of 1-2% NO_3 between samples from the same premise. In one extensively investigated case, 23 of 50 calves turned into a ryegrass pasture containing 6.6-8.9% KNO_3 (4-5.3% NO_3) died within a 12-hour period.[359] In another instance, calves died if left on a ryegrass pasture containing 3.6% NO_3 for more than one hour.[360] A dose of NO_3, later calculated to be 170 mg NO_3/kg BW, from contaminated hay killed seven of 200 heifers. The herdsman tried to dilute the toxic hay in half and killed seven more.[361] The authors speculated that concurrent overfeeding of monensin enhanced ruminal reduction of NO_3^- to NO_2^-, thus potentiating the toxicity of the hay. Harris and Rhodes[362], summarizing the experience of farmers during a severe drought in Victoria, Australia, reported several hundred animals were killed by plants containing "over 1.5% NO_3." Three cows fed hay containing 1% NO_3 died within 30 minutes.[353] Eleven cows aborted, and 73 of 153 died when fed sudax hay containing 1.1-3.1% NO_3.[363] McKenzie summarized several cases with acute

mortalities of 16-44% on button grass (2.4-7.2% NO_3) grown in N-rich soil in Queensland, Australia.[349] Animals grazing the same grass in adjacent paddocks without the extra N were unaffected.

Although not as common as poisoning from feedstuffs, contaminated water has resulted in acute poisoning, including abortions and death. Seven of 12 cows died shortly after drinking water containing 2,790 mg NO_3^-/L.[336] Several authors reported lethality as a result of fertilizer-contaminated water (1,000-6,000 mg NO_3^-/L).[333,351,364] Contaminated liquid whey, fed in addition to water and containing 2,200-2,800 mg NO_3^-/L, killed 17 of 360 cattle. Whey containing only 400-800 mg NO_3^-/L did not kill any animals, but it did result in 26 of 140 cows aborting.[333] Yong et al.[334] reported that water, contaminated with 4,800 and 7,000 mg NO_3^-/L as a result of blasting water holes, killed 16 of 100 and four of 90 cows in two separate incidents in Saskatchewan, Canada.

It is known the NO_2^- ion may react with secondary amines (common in many foodstuffs) under conditions typical of the adult human stomach (pH 1-4) to form nitrosamines.[365-367] Many nitrosamines are potent animal mutagens and carcinogens. Bacterial reduction of NO_3^- to NO_2^- does not occur under the acid conditions necessary for nitrosamine production, or *vice versa*, but it is theoretically possible NO_2^- excreted in saliva or ingested in water might cause cancer in people. In practice, however, salivary secretion contributes much less NO_2^- than other sources such as vegetables, and attempts to link nitrosation, mutagenesis, and/or cancer with drinking water NO_3 consumption have been negative or only equivocal.[365-371] This, together with the fact most herbivores have GI conditions that are even less prone to nitrosamine formation than humans, suggests cancer is not a likely sequella of NO_3 exposure in our species of interest. Elevated NO_2 *is* a potential acutely toxic hazard, however. Four of four sows were killed by drinking 1,940 mg NO_2/L water.[335] Nitrite is reported to be 2.5 times more toxic than NO_3 in ruminants and 10 times more in monogastrics[317], and the minimum toxic dose is reportedly between 20-90 mg NO_2/kg BW in pigs and 90-170 mg NO_2/kg in cattle and sheep.[313,358]

Chronic NO_3 poisoning is another area of controversy. Mice exposed to 1,000 mg NO_3^-/L in drinking water for 18 months (life time) died prematurely starting at 17.5 months. The result was of only marginal statistical significance, and no possible mechanism for the result was proposed.[343] Mice exposed to 100 mg NO_3^-/L showed no effects in any parameter measured (liver function, kidney function, serum protein, etc.). Seerley et al.[344] fed breeding gilts $NaNO_3$ in water to provide 1,320 mg NO_3^-/L for 105 days with no effect. Similar results were reported for weanling pigs.[372,373] Fan et al.[374] reviewed the veterinary literature on chronic NO_3 toxicity and concluded it "failed to provide evidence for teratogenic effects attributable to NO_3 or NO_2 ingestion." A retrospective epidemiologic study of pregnant women in the Mt. Gambler region of Australia indicated a "statistically significant increase in risk of bearing a malformed child" in women who drank water with more than 66 mg NO_3^-/L, but it did not take into account other factors associated with the water wells. Bruning-Fann et al.[375] surveyed water from 712 swine operations in the United States and found no differences in litter size or piglet mortality attributable to well-water containing NO_3^- (1-443 mg/L).

After a widespread drought in the American Midwest in the mid-1950's, several authors summarized the experience of multiple field investigations.[376-378] Purportedly, feed concentrations greater than 0.5% NO_3 or water supplies containing more than 500 mg NO_3^-/L were hazardous to cattle fed "poor quality" rations. Case[377] was first to propose that NO_3 interfered with vitamin A metabolism. The results of many controlled experiments since then have rendered this theory "questionable".[312] Sheep fed 2.5% $NaNO_3$ (approximately 1.75% NO_3) diets for 135 days had slightly lower liver vitamin A concentrations than controls, and gains were depressed. A second replicate of the same experiment did not exhibit decreased vitamin A nor was there an increase in metHb or signs of toxicity in either group.[348] Fourteen yearling steers were divided into seven groups and treated with various combinations of NO_3^- in drinking water, NO_2^- in drinking water, *E. coli*, and a "thyroid depressant." Creative use of statistics demonstrated depressed carotene utilization, but there were no other effects.[379] On the other hand, heifers fed various amounts of NO_3 up to 0.9% NO_3 in diets containing 20% or 40% concentrate did not exhibit any difference from controls in carotene conversion or hepatic retinol concentrations.[356] Feedlot cattle fed 0.81% dietary NO_3 as $NaNO_3$ exhibited poor gains as a result of decreased feed consumption. Gains were not improved by supplementing with 12,000 IU vitamin A.[357] Emerick[380] reviewed the literature and concluded that chronic effects involving vitamin A, thyroid function, and other hypothetical chronic mechanisms only occurred at doses that were nearly toxic due to metHb formation.

Winter and Hokanson[381] fed varying amounts of NO_3 (330-690 mg/kg BW) to dairy heifers as part of their ration to maintain metHb levels at either 25-30% or 50% for the last six months of pregnancy. One animal aborted, possibly as a result of NO_3 intoxication and two died of acute poisoning after a diet change, but no chronic effects could be ascertained. Crowley et al.[332] in what, to date, has been the most rigorous experimental attempt to produce chronic NO_3 poisoning in dairy cattle, fed high NO_3^- water (384 mg NO_3^-/L) for 35 months with no effects on conception rate, twinning, stillbirths, abortions, retained placenta, or a variety of production parameters. The only statistically significant effect was a slightly lower first-service conception rate in the NO_3 group. The authors concluded that, in a herd fed a balanced ration, "water containing up to 400 ppm NO_3 should not cause any serious problems."[332] Ensley[382] attempted an epidemiologic approach to the question of high NO_3 water for dairy cattle. In a survey of 128 Iowa dairies with water concentrations from 1-300 mg NO_3^-/L, he found water NO_3 concentrations were positively correlated with services per conception, which agrees with the results of Crowley et al., but several other potentially confounding factors such as the size of the farm and other contaminants in the water were also positively correlated with NO_3 concentrations.

Other attempts to produce chronic NO_3 poisoning in ruminants have been unsuccessful. Sinclair and Jones[327] dosed ewes with 15 g KNO_3 (similar to 1.5% in forage) for two months and then sprayed the same dose of NO_3 on the daily hay ration for another seven weeks. Ewes were fed diets containing 0.2-2.6% NO_3 as $NaNO_3$ or from natural sources for 12 weeks with no effects on health or pregnancy.[340] Despite elevated serum NO_3^- concentrations, there were no effects on metHb, body condition, or reproduction in the treated group. Whethers fed $NaNO_3$ in drinking water to provide 1,465, 2,900, or 4,400 mg NO_3^-/L for 84 days did not differ from controls in gain and water consumption, and only modest increases in metHb concentrations were seen at the highest dose.[344] Feeder lambs fed 3.2% dietary KNO_3 (1.9% NO_3) until slaughter differed from controls only in "carcass quality[326]." Emerick[380] reviewed the literature in 1974 and concluded that feeds containing less than 0.44% NO_3 and water with less than 440 mg NO_3^-/L were "well within a safe range for all classes of livestock."

Summary

There is no question NO_3 contamination of drinking water can result in acute death and/or abortion in ruminant livestock. Cattle are usually reported to be more susceptible than sheep, with monogastrics such as horses and swine being relatively resistant. Surprisingly, we were able to find only one report of NO_3 poisoning (from feedstuffs) in wild ruminants[383], but, given the physiological similarities with domestic animals, it is reasonable to assume deer, antelope, and elk are also susceptible.

The chronic toxicity of very low doses of NO_3 is controversial. Despite repeated attempts (and failures) to reproduce vitamin A deficiency, hypothyroidism, or other chronic forms of NO_3 toxicity, experimentally it does not seem that dietary concentrations significantly less than those required for acute intoxication cause measurable ill-effects in domestic ruminants. While there is no question NO_3 can produce abortions in ruminants, the dose required appears to be very near that required for acute toxicity. The most scientifically rigorous examination of chronic NO_3 toxicity to date[332] concluded that water concentrations less than 400 mg/L (the concentration tested) should not pose any hazard to a well-managed herd.

The lowest toxic dose of NO_3 in cattle in the experimental studies we reviewed is somewhat less than 200 mg NO_3^-/kg BW, although there were several experiments that failed to produce any effect at considerably higher (as much as 800 mg/kg BW) doses. Clinical (i.e. anecdotal) reports, in particular those of Yeruham et al.[384] and Slenning et al.[361], push the minimum toxic dose down to near 100 mg NO_3/kg BW. There are some uncertainties associated with these two reports. Yeruham did not specify the amount of toxic whey consumed (we assumed 20% BW when figuring a dose as it occurred in a hot climate), and there was a two-fold variation in analytical results between samples. Slenning et al. suggested other factors, notably overfeeding an ionophore, might have potentiated the toxicity of NO_3. The next lowest concentration reported to be acutely toxic was 1% NO_3 in *Chenopodium* hay, which would provide approximately 250 mg NO_3/kg BW in cattle under the assumptions outlined in the Introduction. Nitrate in water is additive with NO_3 in feedstuffs, with a given dose in water being somewhat more potent than in feed because it is consumed more rapidly.

Assuming negligible forage NO_3 concentrations, a water NO_3 concentration of 500 mg NO_3^-/L (measured as NO_3^- ion) would provide 100 mg/kg BW, which would provide a two-to-three fold margin below the 200-250 mg/kg BW dose above. If forage concentrations are higher (not a rare occurrence in the Great Plains) the permissible water concentration should be adjusted downward.

The NO_2^- ion is commonly described as approximately 2.5-fold more toxic than the NO_3^- ion in ruminants (10-fold more toxic in monogastrics), which implies a safe threshold of about 200 mg/L. We were, however, unable to find sufficient experimental studies or well-documented field investigations upon which to base any conclusion about maximum safe concentrations. This is probably due to the fact NO_3^- is the more stable form of the two in surface waters and feedstuffs, and NO_2^- is only rarely present in negligible concentrations. Garner describes the minimum lethal dose of NO_2 in swine (the most sensitive species) as 40 mg/kg BW[858], which translates to 200 mg NO_2^-/L in drinking water under very conservative assumptions. Intravenous administration (the most potent route of exposure for most toxicants) of 12 mg NO_2^-/kg BW to cattle and 17.6 mg NO_2^-/kg BW to sheep did not produce any reported toxic effects.

Obviously, this is an area that needs further research, but we believe, based upon the existing knowledge base, 100 mg NO_2^-/L (as the nitrite ion) should not cause poisoning in livestock.

7 pH

pH is defined as "the negative log of the hydrogen ion (H^+) activity," although "concentration" is often substituted for "activity" in a working definition.[385] In other words, if the pH is 7.0, the H^+ concentration ($[H^+]$) is 10^{-7} moles per L; if the pH is 3, the concentration is 10^{-3}. Water with a pH below 7 is "acidic," and water with a pH above 7 is "basic" or "alkaline." The definition of "normal" water pH varies between authorities, but it is usually described as somewhere between 5.5-6.5 and 8-9. By comparison, vinegar is pH 3, colas are between 3-4, beer is between 4-5, and milk is around pH 6.4. A related measurement, alkalinity, is based upon the capacity of a water sample to resist a change in pH and is usually reported as mg/L of $CaCO_3$. The pH controls solubility and concentrations of elements in water. For example, many metals precipitate out of alkaline water, whereas many dissolve in acidic water.

Water pH impacts the effectiveness of various water treatments and its palatability for animals. For example, the effectiveness of chlorination is reduced at a high pH. Low pH may precipitate or inactivate medications commonly delivered in drinking water. Sulfonamides are a particular concern in this respect as precipitated medication may leach back into the water after treatment has ended, contributing to potential drug residues in food animals. Acidic water tends to have a sour taste; basic is described as metallic. The taste threshold for hydrochloric acid is as low as 0.0001 mol/L.[385] Acid water also tends to dissolve metals from plumbing and soil it contacts, further impacting palatability.

Function

In the body, extracellular fluid (ECF) is normally maintained within a very narrow range centered around pH 7.4.[385,386] A number of critical physiological processes are pH-dependent, thus any significant departure from "normal" may be harmful to the organism.[386]

Metabolism

The acid-base balance in mammals represents a dynamic equilibrium between metabolic acid production and its elimination. As such, it is influenced by a number of interdependent processes, especially respiration and urine production, chemical buffering by bone and other tissues, and several dietary elements. A comprehensive review of these processes is beyond the scope of this report, but, in brief, a decrease in plasma pH from normal (acidosis) stimulates increased respiration, decreasing the blood concentration of CO_2 that would otherwise react with water to form carbonic acid (H_2CO_3). It also stimulates the kidney to produce urine that is more acidic. It does this by increasing elimination of the H^+ ion. The net result is an active elimination of acid and an increase in plasma pH back towards normal.[385,386] The H^+ ion may also react chemically with buffering molecules in bone, minimizing the magnitude of any pH change.[387,388]

Certain nutrients, referred to as "strong ions," while not acidic or basic in the chemical sense, influence animals' acid-base balance by shifting the equilibrium of internal homeostatic processes to enhance or inhibit the elimination of H^+ from the body. For example, diets rich in Na^+ and K^+ tend to "push" the body in the alkaline direction, whereas Cl^-, as ammonium chloride (NH_4Cl), is used clinically to move the balance in an acidic direction. Dairy nutritionists compound rations on the basis of the "strong ion difference" equation $((Na^+ + K^+) - (Cl^- + S^{2-}))$.[386] Diets with a negative strong ion difference produce mild acidosis shortly before lactation, which decreases the incidence of milk fever.[389-391]

Toxicity

Excessively acid or basic drinking water can theoretically affect animals in four ways. First, extremes of pH may result in tissue damage to the mouth and oropharynx, causing irritation and refusal to drink. Second, unusual extremes of pH may dissolve materials from pipes, ditches, etc., which are toxic or impart an unpleasant taste to the water. For example, Cu, Fe, and Pb concentrations have all been shown to increase in acidic water as a result of leaching the metals from plumbing.[392-395] Consumption of acidic drinks *may* dissolve dental enamel and weaken teeth.[396,397] Finally, consumption of a large amount of excessively acid or basic water could theoretically shift the body's acid-base balance.

We were unable to find any reports detailing acute toxicity as a result of drinking extremely acid or basic water, although co-author Merl Raisbeck has investigated field cases in which cattle drank extremely basic solutions (pH 12-14) resulting in erosions and hemorrhage in the mouth and esophagus.

"Acid rain," precipitation rendered acidic by atmospheric pollution, is a well-recognized problem in aquatic organisms because toxic elements, especially Al, are leached from solids that come in contact with the acidic water. It is especially problematic in poorly buffered surface waters of northeastern North America.[398] Mammals are considerably less sensitive to the effects of dissolved metals than fish, but acidic water supplies have been suggested as contributing to the presence of Pb and Cu in domestic drinking water, a possible concern for human health.[392,393,395] Even if not present at toxic concentrations, many elements impart a repellent taste to water.

Despite the hypothetical potential of acidic (pH < 5.5) water to cause acidosis in animals, water systems in laboratory animal colonies and, to a lesser extent, swine facilities, are commonly acidified to minimize bacterial infections.[396,399-401] It is thought the acidified environment protects the intestinal epithelial barrier from bacterially-mediated disruption as well as reducing bacterial contamination in the water itself.[399]

Some effects have been observed in ruminants and monogastrics due to the ingestion of acidic feed and water. Acidogenic rations are fed to dairy cattle during late pregnancy to prevent milk fever. For example, the addition of NH_4Cl and ammonium sulfate ($(NH_4)_2SO_4$) at 98 g apiece/head/day to the diet of lactating dairy cows lowered blood pH, increased blood calcium (Ca^{2+}), and increased excretion of urinary Ca^{2+} and the ammonium ion (NH_4^+).[390] The same amount of these salts added to drinking water would result in a pH of approximately 5.5. Mineral acids (HCl and H_2SO_4) have been fed to prevent milk fever.[402] Dairy cows given rations containing 0.65% or 1.8% hydrochloric acid (HCl) (equivalent to pH 2-3, respectively, if added to drinking water) had increased blood Ca^{2+}, a small (0.05) decrease in blood pH, acidic urine, and a decreased incidence of milk fever.[391] Rats given water acidified to pH 2.0 with HCl or H_2SO_4 for six weeks exhibited somewhat decreased feed intake and water intake, and weight loss compared to those fed pH 2.5 or pH 7.0 water; however, stomach pH values were not statistically different amongst treatment groups.[403] Rats consuming water acidified to pH 2.5 with HCl for six to 11 months showed no changes in weight and "negligible" damage to tooth enamel.[397] Rats drinking water with pH 2 for 21 weeks drank slightly less than controls, while those consuming pH 3 water showed no measurable effects in any health parameter.[404] Water with a pH of 2.5 given to rats for 30 days resulted in decreased water intake, while water with pH 3 given to rats produced no growth deficits.[396] Rats drinking water with a pH of 1.4-3.5 for 42-84 days showed accelerated erosion on the tooth surface; however, oral cavity pH was unchanged.[405] Mice consuming acidified water (pH 2.0) for 120 days had slightly decreased reticuloendothelial clearance rates when compared to controls, possibly as a result of decreased growth and smaller spleens.[406] It is questionable if the physiologic effects in these reports are a direct result of acidic water on systemic acid-base status, *per se*. Even a study that reported some relatively subtle effects (decreased gains) in rats at pH 2.5 or less, gastric pH was unchanged,[403] and rats and rabbits fed pH 2.3-2.5 water for seven months maintained normal blood pH.[401]

Several experiments reported no effects when acidic water was fed, and it is commonly believed that acidifying water to approximately pH 3 is beneficial in rodent colonies. Rats consuming water with pH of 2.5 gave birth to and weaned more pups than control animals.[407] Rats and rabbits ingesting water at pH 2.3-2.5 for seven months showed no affect on health as measured by growth and multiple blood parameters.[401]

Summary

Existing human drinking water standards (pH 6.5-8.5) were established decades ago for aesthetic reasons (especially taste) and to protect plumbing from corrosion, rather than upon health-based criteria.[392,408] Although a number of Cooperative Extension Service Web sites suggest water below this range will produce pathologic acidosis in cattle, none we examined offered hard evidence. Nor were we able to find any references to direct health effects of moderately acidic or basic water in animals. There *are* a number of references to the beneficial effects of acidifying laboratory animal and swine water with mineral acids to pH 3. The only adverse effects in these reports were relatively subtle and occurred at pH < 3.0. The only example of feeding a pure mineral acid to ruminants (equivalent to approximately pH 2-3) resulted in acidified urine, but there were no adverse health effects over a several week period. There is no equivalent data

for basic drinking water. From a purely pathophysiological standpoint, it seems unlikely that water with a pH between 3.0 and 7.0 would cause health problems in otherwise normally managed animals. An exception might be feedlot ruminants, which are often marginally acidotic as a metabolic consequence of the high soluble carbohydrate rations they receive. In this case, acid water might be sufficient to trigger a crisis.

The other potential adverse effects of basic or acidic water involve mobilization of potentially toxic substances (e.g. metals) from plumbing or soils. While it seems unlikely the amounts mobilized would be sufficient to actually cause poisoning under most conditions, it is quite probable they would be large enough to cause water refusal. Because the effect of any given pH on palatability depends upon what the water contacts, there is no way to make any wide-reaching recommendation in this regard.

We suspect the commonly touted acceptable ranges for drinking water pH (a low of 5.5-6.5 and a high of 7.5-9.0) are excessively conservative from a strictly animal health standpoint, at least on the acid side, but there are not sufficient experimental and/or clinical data to offer a specific alternative.

8 Selenium

Selenium (Se), a metalloid that shares many chemical properties with S, is predominantly present in cretaceous rocks, volcanic material, seafloor deposits, and glacial drift in the Great Plains.[409] It can be present in soil at levels sufficient to cause toxicity or low enough to result in deficiency in grazing animals. Either outcome can result in serious economic losses to livestock producers and illness and/or death in wildlife. Normal soil concentrations range from 0.1 to 2.0 µg total Se per gram of soil; however, in seleniferous areas such as Wyoming, soils can contain as much as 30-324 µg Se per gram of soil.[410] Plants grown on such soils tend to accumulate Se and, depending upon the species, may, in fact, bioconcentrate Se to concentrations in excess of 10,000 ppm.

Water in contact with seleniferous rocks and soils (e.g. irrigation wastewater) may also accumulate Se.[411,412] The most common form of Se in Wyoming surface waters is the selenate (SeO_4^{2-}) ion. "Normal" surface water is described as containing less than 2 µg Se/L[411], and it is thus not normally a major source of Se for livestock and mammalian wildlife; however, poisoning as a result of seleniferous water has occurred in horses and sheep.[413-415] Dissolved Se becomes concentrated in successive levels of the aquatic food chain and is a major concern for waterfowl that depend upon aquatic biota for food[411,416,417]; however, aquatic bioconcentration does not pose a hazard to large herbivores (e.g. cattle, elk) under normal conditions as their intake of algae and aquatic organisms is very small. Interestingly, bioaccumulation of Se in the aquatic food chain actually removes Se from the water column,[411,418] thus decreasing the risk to large mammals. Forages irrigated with seleniferous water contain elevated Se concentrations and can pose a risk to grazing animals.[411,412,418] While each of the preceding sources is important, this report is concerned specifically with the hazard posed to livestock and wildlife by Se in drinking water and will focus upon that source.

Essentiality

Selenium is an essential trace element. Most authorities agree that, worldwide, deficiency is a more common problem than toxicity, and, thus, for the last 40 years, much more research has focused upon the effects of inadequate dietary Se than too much. This research may still be useful for its insight to Se metabolism. Within the body, Se is a component of several enzyme systems, most involved in catalyzing oxidation-reduction reactions.[419,420] The Se-requiring system thought to be most responsible for damage in deficiency situations is glutathione peroxidase.[410,421] Selenium supplementation decreases the incidence of white muscle disease, a degenerative condition in muscle resulting from oxidant stress.[421-423] The FDA permits 0.3 ppm Se (total ration) as a feed additive, up to 0.7 and 3 mg Se per head per day as a feed supplement for sheep and beef cattle, respectively, and in fortification mixtures up to 90 and 120 ppm Se for sheep and cattle, respectively.[424] Deficiency is rarely a problem in Wyoming and northern Great Plains states.[425,426]

Metabolism

Selenomethionine is the main form of Se in common forages, even though it is not a major component of accumulator plant species.[427-429] It constitutes the majority of ingested Se, however, as accumulator plants are highly unpalatable and are usually avoided to the point of starvation.[430] Waterborne Se, usually selenite (SeO_3^{2-}) or selenate (SeO_4^{2-}) ions, normally comprises a relatively small portion of large herbivores' total exposure; however: 1) water concentrations sufficient to cause poisoning have been recorded in Wyoming[431,432]; 2) poisoning has occurred in livestock as a result of Se contamination associated with mineral extraction; and 3) Se-contaminated water has the potential to add to the already high background forage concentrations common to many parts of the Great Plains.

Dietary Se is absorbed from the small intestine in both ruminant and monogastric species. Selenocysteine and selenomethionine are transported across the intestinal epithelium by active amino acid transport mechanisms.[433] Selenite is absorbed passively by simple diffusion, but SeO_4^{2-} is accumulated via a Na-mediated carrier with sulfate (SO_4^{2-}).[419,434,435] To date, there is no evidence for homeostatic control of Se absorption as neither dietary Se concentration nor bodily Se status influences absorption

efficiency.[436,437] Absorption of Se *is* influenced by animal species and the form of Se ingested. Selenomethionine, the predominant form of Se in forage plants, is absorbed more efficiently than inorganic forms of Se, at least when the comparison is based upon tissue concentrations.[438] Selenate is better absorbed than SeO_3^{2-}, at least in laboratory rodents, and both are more efficiently absorbed than elemental Se.[419] Ruminants reduce Se to unabsorbable selenides in the rumen and are therefore, to some degree, protected against poisoning. It is not unusual to see selenosis in pastured horses, while cattle on the same pasture remain unaffected.[426] Because the reduction of Se by rumen microflora is heavily influenced by other dietary factors, ruminants also exhibit greater variation in Se absorption than monogastrics.[415,439]

Following absorption, Se becomes associated with plasma proteins, mainly albumin and selenoprotein-P, for transport to tissues.[440] Selenomethionine is non-specifically substituted for methionine in protein[437] and only becomes available for either toxicity or nutrition as the protein turns over. Other forms are incorporated into essential selenoproteins and/or methylated to dimethylselenide and the trimethylselonium ion for elimination.[441] Both elimination and protein incorporation appear to involve metabolic activation to a reactive intermediate, which, when cellular defenses become saturated, is responsible for most of the toxic effects of Se.[419] Under normal conditions trimethylselonium is eliminated via urine and, to a lesser extent, bile, but as tissue concentrations increase to toxic levels an increasing percentage is eliminated via respiration as dimethylselenide.[419] Increased (i.e. potentially toxic) dietary Se also results in a shift of the distribution of Se between various proteins and body compartments[411,438,442] although both the physiologic and toxicologic significance of this observation is yet to be elucidated.

Selenium interacts with a number of other common dietary constituents, primarily at the pharmacokinetic (uptake, distribution, and elimination) level. These interactions modulate both the nutritional and toxic properties of Se. Arsenic (As) decreases Se toxicity by decreasing tissue Se concentrations.[35,38,443] Mercury decreases the toxicity of Se in birds[37,435] and possibly marine mammals.[411] Sulfur is thought to alleviate Se toxicity.[444] When sheep were given 2 mg of Se as sodium selenate (Na_2SeO_4), sheep receiving 0.05% dietary S showed a greater degree of Se toxicity when compared to sheep given 0.11, 0.17, or 0.24% S, mostly as added SO_4.[445] Dietary SO_4 decreased the Se balance in dairy cows fed Na_2SeO_4, reduced the true availability of nutritional levels of Se, and increased its excretion via feces in dairy cattle.[446] This interaction is hypothesized to occur as a result of S decreasing ruminal pH, altering Se metabolism to unavailable forms, as well as reducing the incorporation of dietary Se into ruminal bacteria.[445,447] Ruminal interactions, however, are not the only explanation for the inhibitory effect of S on Se toxicity. Added dietary SO_4 (0.29%) resulted in a 20% growth increase in rats fed 10 ppm Se; 0.58% SO_4 caused a 40% growth increase; and 0.87% SO_4 slightly prevented liver damage due to Se intoxication.[448] The interaction between SO_4 and Se is not universal, however. Three thousand mg SO_4^{2-}/L in drinking water failed to alter the uptake of sub-lethal dietary concentrations of sodium selenite (Na_2SeO_3) or selenomethionine in mallard ducks,[449,450] and more research is needed to elucidate the conditions under which the protective interaction occurs. Cyanogenic glycosides block uptake of Se and ameliorate Se toxicity[242,451] and have been investigated as a means of protecting livestock against selenosis. Copper and Cd have also been shown to reduce Se toxicity, though the mechanisms are not known.[37,452] Increased dietary protein has been suggested to reduce the severity of poisoning.[453] The type of feedstuff (alfalfa vs. grass hay) may influence the bioavailability of Se.[242] Even being Se deficient predisposes animals to Se toxicity.[454]

Toxicity

Although essential, Se exhibits a very narrow margin of safety. Toxic effects have occurred in livestock at dietary concentrations only 40-100 times larger than deficiency.[421] The form of Se administered influences tissue accumulation and thus toxicity.[455-460] The chemical species most common in water are the inorganic ions SeO_3^{2-} and SeO_4^{2-}. Selenium toxicity can be manifested in two forms, acute or chronic. Acute toxicity usually results in GI, liver, kidney, and heart damage, shock, and sudden death.[430,459,461] Selenium has also been implicated as a cause of hypertension and cardiac damage.[462] Reproductive deficits, including teratogenesis and embryonic mortality, occur in avian species[411,417,456,463]; however, there is no evidence Se is teratogenic in mammals.[464-466] Selenium may compromise reproduction in mammals.[467,468] Chronic selenosis ("alkali disease") in ungulates is manifested most obviously as epithelial damage (hair loss and cracked hooves) as a result of necrosis of the keratinocytes[450,469] and ill-thrift.

A single subcutaneous dose of 2 mg Se/kg BW given to pigs caused death after four hours.[470] Three hundred seventy six of 557 calves injected with a Na_2SeO_4 solution that contained 100 mg Se (0.5 mg/kg BW), rather than the 12 mg Se that was intended, died within five weeks.[471] Intramuscularly injecting 2 mg Se/kg BW as Na_2SeO_3 into four calves caused shock within three hours and death in 12 hours.[472] Ewes injected intramuscularly with doses from 0.4-1.0 mg Se/kg BW exhibited dyspnea, anorexia, colic, and a seromucoid nasal discharge. Doses less than 0.6 mg/kg BW were not lethal within 192 hours. The calculated LD_{50} was determined to be 0.7+/-0.035 mg/kg.[473] Injecting sheep intramuscularly with 0.68 mg Se/kg BW as Na_2SeO_3 produced death within 20 hours. Smaller doses took longer to be lethal, or they only caused reduced feed consumption and transient signs of intoxication.[473] Ninety µg Se/kg BW, given intramuscularly as Na_2SeO_4, produced no clinical signs in ewes. Three hundred fifty µg Se/kg BW was lethal in some ewes. Barium selenate ($BaSeO_4$), given similarly at rates of 0.225, 0.454, 0.908, 1.816, or 4.9 mg/kg BW produced no signs other than irritation at the injection site.[474]

Selenium fed as Na_2SeO_3 at rates of 22.3, 33.5, or 52.1 ppm dietary Se to rats for 359 days resulted in the deaths of five of nine, eight of nine, and all nine rats in the three treatment groups, respectively, as well as decreased fertility and anemia in the population as a whole.[475] An experiment focused on Se as a causative factor of tooth decay offered drinking water containing 3 mg Se/L as Na_2SeO_3 to rats for four weeks. Two of 15 rats died, and the others suffered significantly reduced feed consumption, water intake, and body weights.[476] Rations containing 6.4 ppm Se, as seleniferous wheat or Na_2SeO_3, resulted in significantly decreased growth and enlarged spleens in rats. Lower concentrations were without measurable effects. Higher concentrations resulted in decreased organ weights and anemia and were lethal in some rats.[477]

In one of the earliest controlled studies of Se dose-response, Miller and Williams[478] reported the single *oral* "minimum fatal doses" (which they defined as a dose large enough to kill 75% of the test group) were 0.68 mg Se/kg BW for horses and mules, 1.1-2.2 mg Se/kg BW for cattle and 2.2-3.6 mg Se/kg BW for swine, all given as Na_2SeO_3. Although the experimental protocol was not up to modern standards, more recent reports in cattle and horses usually agree within two- to five-fold. Depression, anorexia, hind limb ataxia, and sternal recumbency developed in 256 pigs in a commercial piggery after the pigs were exposed to feed containing 84 ppm Se.[479] Feed containing 8.1 ppm Se resulted in decreased feed consumption and paralysis in 54 feeder pigs.[480] Rations containing 14.75 ppm and 26.65 ppm selenium caused hair loss, reddening of skin, and hind limb ataxia in 80 of 160 pigs. Higher concentrations resulted in feed aversion.[481] Porcine diets fortified with approximately 25 ppm Se as Na_2SeO_3 resulted in hair and hoof lesions and weight loss. Higher concentrations produced feed aversion and poliomyelomalacia.[482] Corn, naturally contaminated with 10 ppm Se, fed for five months, resulted in two of five pigs developing alopecia and hoof lesions.[483] Pelleted rations containing 26.6 or 31.7 ppm Se, fed as Na_2SeO_3 or the Se-accumulator *A. bisulcatus*, respectively, and fed for several weeks, resulted in reduced feed consumption, alopecia, cracked hooves, and paralysis in pigs.[457]

A single dose of 5 mg Se/kg BW, given orally as Na_2SeO_3, was acutely toxic in lambs.[484] Drenching 190 lambs with 6.4 mg Se/kg BW resulted in the death of 180 within 15 days.[485] Twenty lambs, four to 14 days of age and averaging 4.5 kg, were mistakenly given 10 mg Na_2SeO_3 (2.2 mg Se/kg BW) orally to prevent white muscle disease; seven died within 17 hours, and eight more experienced diarrhea as a result of acute toxicosis. As a follow-up experiment, an additional ewe was injected with 0.45 mg Se/kg BW, which resulted in death within eight hours.[486] Glenn et al.[464] fed ewes increasing dosages of Na_2SeO_4 for 100 days and concluded the "minimum lethal" dose was 0.825 mg Se/kg BW/day. One of a group of 12 adult ewes and their lambs, pastured in an area of seleniferous forage and water, died following 14 days; other animals were unaffected when removed after four weeks. The exposure from the combination of contaminated forage and water was calculated to be 0.26 mg Se/kg BW/day.[413] Sheep were lethally poisoned by grazing high Se forage (<49 ppm Se DM) and drinking high Se water (340-415 µg Se/L) for four weeks; however, a similar group on a neighboring pasture with forages < 13 ppm Se and normal water were unaffected.[413]

Steers gavaged with varying doses between 0.25 and 0.5 mg Se/kg BW as Na_2SeO_3 exhibited inappetence, depression, and death. Two of eight died within eight weeks, and four more died within 14 weeks.[487] Feeding 0.28 and 0.8 mg Se/kg BW (approximately 10 ppm and 25 ppm Se, respectively, under range conditions) as selenomethionine or 0.8 mg Se/kg BW as Na_2SeO_3 to yearling steers for four months resulted in overt clinical signs of alkali disease in one steer and histological lesions of dyskeratosis in several more.[458] Primary antibody response was

significantly impaired in the same dose groups.[488] A dose of 0.15 mg/kg BW (approximately 5 ppm Se in diet), fed as either selenomethionine or Na_2SeO_3, and 0.28 mg/kg BW as Na_2SeO_3 in the same experiments were without measurable effects.[458,488] Dosing calves with 0.25 mg Se/kg BW as Na_2SeO_3 via bolus for 16 weeks resulted in hair and hoof lesions typical of selenosis and indications of increased oxidant stress.[489] Decreased growth and anemia occurred in pre-ruminant calves fed 10 ppm as Na_2SeO_4 for 40 days, but no measurable effects occurred in calves fed 0.2-5.0 ppm Se.[490]

There is a relative paucity of *quantitative* data in horses, although horses are the species most commonly diagnosed with selenosis at the Wyoming State Veterinary Laboratory. Ponies dosed via stomach tube with 6 or 8 mg Se/kg BW as Na_2SeO_3 developed signs of acute selenosis within six hours; ponies given 2 or 4 mg Se/kg BW remained clinically normal for the 14-day experiment.[452] A 4-year-old gelding accidentally given 25 mg Se (0.055 mg Se/kg BW) orally as Na_2SeO_4 for five consecutive days developed a straw-colored exudate on its lips, prepuce, and anus. Within three weeks, its hooves had separated from the coronary band, and alopecia was present on the mane and tail.[491] This report did not investigate the Se content of the rest of the diet. Chronic Se toxicosis was diagnosed in seven horses consuming hay containing between 0.49 and 58 ppm Se (mean 22 ppm) in combination with a mineral supplement that contained 1.9 ppm Se.[492,493] Eight of 20 horses developed alkali disease after being fed alfalfa hay that contained 5.9-17 ppm (mean = 11.9 ppm) Se for several weeks.[494] Several horses were diagnosed with alkali disease in southeastern Idaho after being turned into a pasture where the only water supply contained approximately 0.5 mg Se/L.[495] Assuming water consumption of 10% BW, this works out to 0.05 mg/kg BW, but there was probably some additional Se from forage in the pasture. Feeding horses oats containing 96 ppm added Se (19.2 ppm of total ration) as Na_2SeO_3 for several months resulted in listlessness, loosening of hair, softening of the horny wall of hoof, and, ultimately, death.[496] A 9-year-old gelding developed a swollen prepuce, vesicles on its nostrils and mouth, and hoof lesions of alkali disease after receiving 153.4 mg Se per day in his feed (16.4 ppm DM) for several days.[497] In Queensland, Australia, horses and sheep were diagnosed with chronic and acute Se toxicity after grazing areas with vegetation containing 200-3,038 ppm Se and corresponding soil concentrations of 64-128 ppm Se.[498]

In goats, daily oral doses of 5-160 mg Se/kg BW administered via capsules were uniformly lethal within 31 days. Daily doses of 0.11-0.45 mg Se/kg BW were given for 225 days with no toxic effects.[499] Elevated levels of selenium in the soil (1.45-2.25 ppm Se) and forage (40.32-80.64 ppm Se) in West Bengal, a state in India, have been blamed for a gangrenous condition ("Deg Nala") in grazing buffaloes exhibiting hoof cracks, malaise, abdominal pain, laminitis, and edema on the tails and ear tips.[500] Feeding captive pronghorn antelope grass hay that contained 15 ppm Se for 164 days did not result in any overt clinical signs but did decrease primary antibody response to a challenge with hen egg albumin.[501]

The foregoing notwithstanding, feeding steers seleniferous wheat, seleniferous hay, or a control diet with supplemental Na_2SeO_4 to supply 12 ppm dietary Se (65 µg Se/kg BW/day) for 100 days did not produce any *obvious* evidence of selenosis.[442,502] Ellis et al.[503] fed adult Holstein cows up to 50 mg Se/head/day (approximately 87 µg Se/kg BW/day) as Na_2SeO_3 for 90 days, followed by 100 mg Se/head/day (approximately 175 µg Se/kg BW/day) for another 28 days with no apparent health effects. Ewes tolerated 24 ppm dietary Se from Na_2SeO_3 or 29 ppm Se from *A. bisulcatus* for 88 days[504] or 10-15 ppm dietary Se through two pregnancies.[505] Cristaldi et al.[506] fed wether lambs Na_2SeO_3 at dietary Se concentrations up to 10 ppm for one year with no effect on rate of gain, serum enzymes, or pathology and concluded "≤10 ppm dietary Se as selenite is not toxic." The same research group fed 4, 8, 12, 16, and 20 ppm dietary Se as Na_2SeO_3 to adult ewes for 72 weeks with no effect upon body weight nor other evidence of poisoning.[507]

Summary

Although the NRC[421] suggests that horses are about as sensitive to oral Se as cattle, sheep, and goats, our research indicates that species sensitivity is horses > cattle > sheep and goats. Experience at several regional diagnostic labs indicates horses may be poisoned while ruminants using the same forage and water remain unaffected.[426,508,509] With the exception of one study in antelope, there is insufficient dose-response data upon which to base safety recommendations in large mammalian wildlife. That said, there are reports of elk and deer sharing pastures with horses, sheep, and cattle, where the horses developed alkali disease, without any measurable ill-effects in the elk and deer.[510] *Thus, water that is safe for horses should be safe for other livestock and ruminant wildlife species.*

The effects of water-borne Se are, like many other elements, additive with feed content. The chemical form of Se in surface waters is predominately SeO_3^{2-} or SeO_4^{2-}, which is fortunate as these ions are the forms most thoroughly researched. In theory, relatively small concentrations of Se in water may be sufficient to push animals on moderately high Se forages over the edge of toxicity. Unfortunately, the Se content of forage and hay in this region varies from marginally deficient to downright toxic, and other dietary factors such as protein, vitamin E, or cyanogenic glycosides may modify the effects of a given concentration of dietary Se. For purposes of this report, we assumed a "typical" forage containing 1 ppm Se (mostly as selenomethionine), normal protein, vitamin E, and other trace element concentrations. The threshold for chronic poisoning in horses from the literature is 0.05-0.1 mg/kg BW/day. This agrees with unpublished observations from our laboratories. Thus, water that contains 0.25 mg Se/L, consumed at a rate of 10% BW, combined with "average" Se forage, would constitute a potentially hazardous dose. In extremely hot weather, working horses drinking 20% BW of 0.125 mg Se/L water (a *very* conservative assumption) would receive a hazardous dose.

In areas where forage Se concentrations are higher, or if horses are receiving dietary supplements that contain Se, safe water concentrations will have to be adjusted downward, but under normal conditions, 0.1 mg/L should not cause problems.

9 Sodium Chloride

Sodium chloride (NaCl), or salt, was one of the first nutrients to be identified as essential to life. In ancient times, city locations were based upon the availability of salt, water, and food.[511] Although ocean water contains approximately 2.68% NaCl and animals utilizing this environment face the challenge of coping with excessive salt, most terrestrial animals find it difficult to obtain adequate dietary salt and have developed methods to conserve NaCl.[512-514] Due to the relative scarcity of salt in typical pastures, thousands of metric tons of NaCl are produced each year for use in mineral supplements.[511] Sodium (Na) and chlorine (Cl) are rarely found in elemental form in nature; however, most of the toxic effects of NaCl are due to Na. When appropriate, articles that mention one or the other separately will be cited.

Essentiality

Both Na and Cl are essential elements for practically all forms of life. Sodium (Na^+) is the major extracellular cation while Cl^- is the major extracellular anion; together, they are responsible for maintaining acid-base balance and regulating the osmotic pressure of bodily fluids.[515,516] Excitable cell membranes (e.g. nerve and muscle cells) depend upon tightly regulated Na^+ and Cl^- concentrations in cells and the extracellular fluid (ECF). Blood, a specialized form of ECF, consists of approximately 0.9% NaCl.[514] Sodium chloride is reportedly the only mineral animals truly crave and will actually seek out.[514,517] The dietary NaCl requirement of swine is between 0.10 and 0.14%, and 0.18% Na is needed to achieve optimal performance.[518-521] Similarly, the optimal dietary Na concentration in horses is 0.16-0.18% DM[515] and for cattle is 0.08-0.1%.[522] Under extreme conditions, such as high temperatures, lactation, or hard work, these requirements increase due to increased excretion of both Na^+ and Cl^-.[523,524] The clinical signs of sodium chloride deficiency are polydipsia (extreme thirst), polyuria (extreme urination), salt hunger, pica (licking foreign materials), weight loss, and decreased milk production.[525,526] Chloride deficiencies have been produced by feeding diets with 0.10% Cl or less and present as anorexia, weight loss, lethargy, decreased milk production, polydipsia, polyuria, and cardiopulmonary depression, etc. as clinical signs.[527-529]

Metabolism

Once ingested, 85-95% of Na and Cl are absorbed in the GI tract, particularly the small intestine. Large amounts of Na and Cl are recycled into the intestinal tract via salivary, pancreatic, and intestinal epithelial secretions, as well as bile. The high intestinal Na^+ concentration is required to transport glucose, amino acids, and other nutrients across the mucosa. Chloride is also secreted into the intestine to aid in creating the low pH environment needed for proteolysis. These secretions must then be reabsorbed further down the GI tract to conserve the elements.[514] It has been suggested that elevated dietary NaCl can increase protein digestion. Hemsley[530] discovered that increased NaCl in the diet increased the rate of passage of solid digesta from the rumen, which decreased microbial degradation within the rumen and increased the amount of protein available in the small intestine. Altered ruminal fatty acid concentrations have also been related to dietary NaCl.[531-533] Dietary Na^+ and Cl^- in excess of physiologic requirements are usually efficiently eliminated from the body via the kidneys.[534]

Potassium is the main intracellular cation, Na is the main extracellular cation, and Cl^- is the main extracellular anion. The relative concentrations of these three elements creates an electrochemical gradient across cell membranes that is essential for nutrient transport, nerve conduction, muscle contraction, and energy generation, and they indirectly aid in maintaining pH balance. Imbalances of these elements result in a variety of disorders from decreased gains to acute death.[535-537]

Toxicity

The toxicity of NaCl is intimately related to the availability of water and is sometimes referred to as "sodium ion toxicity-water deprivation syndrome;" however, if the dose of Na^+ is high enough, Na is toxic regardless of water intake.[538] If adequate water is present, most animals can tolerate relatively large doses by increasing Na^+ excretion.[534,539-542] If sufficient water is not available, acute toxicosis results in dehydration, blindness, incoordination, convulsions, recumbency, and death.[205,538,543,544] The

normal physiological response to elevated dietary NaCl is increased water intake; however, if the water contains high NaCl concentrations, drinking results in even more NaCl intake and increases the risk of receiving a toxic dose.[542] Even when sufficient potable water is available to eliminate excess dietary Na, every gram of NaCl excreted requires producing at least 5 ml of urine[545] at a finite metabolic cost. Thus, slight to moderately elevated Na^+ may impact growth and performance.[542,546,547]

The mechanisms underlying acute toxicity are related to cellular dehydration, or "tissue shrinking," and edema. When extracellular Na^+ concentrations become elevated, water is drawn out of the cell down the concentration gradient, resulting in shrinking.[514,548] Eventually intracellular Na^+ concentrations adjust to a new equilibrium with the extracellular fluid (ECF), and cells return to their normal volume. By two to three days of exposure to elevated Na^+, neurons have replaced the increased intracellular Na^+ with "idiogenic osmoles," hydrophilic, organic molecules that compensate for the attraction of extracellular Na^+ for water.[549] Tissue shrinkage due to elevated ECF Na^+ results in damage to the small blood vessels that supply the superficial portions of the brain. Later, after animals have adapted to elevated extracellular Na^+ concentrations (whether from excessive dietary Na intake or water deprivation) and are allowed free access to water, the extracellular fluid becomes diluted and water is drawn back toward the elevated osmolarity inside the neurons, resulting in cellular swelling and damage. In either case, clinical signs result from cerebrocortical (brain) damage.

Acute toxicity has been observed in cattle, pigs, dogs, and rats after ingestion of extreme concentrations of NaCl. Ninety-one cattle developed muscular weakness, muscle fasciculations, and sternal and lateral recumbency, and they subsequently died, as a result of drinking water from a tank and lagoon that contained 4,370 and 21,160 mg Na^+/L, respectively.[205] Cattle consuming water containing 5,850 mg Na^+/L experienced a 13.7% reduction in body weight, with corresponding reductions in feed and water intakes.[550] Water containing 6,726-6,826 mg Na^+/L resulted in a loss of condition, scouring, and death in 15 of 220 cattle.[551] Six cattle began showing signs of CNS disruption four hours after consuming 50 kg of a supplement containing 50,000 ppm NaCl (19,500 ppm Na), despite drinking water immediately afterward.[538] A pig owner replaced free-choice NaCl as a salt block with two "handfuls" of loose trace mineral salt in 30 gallons of water. Within six days, four pigs began champing jaws, frothing at the mouth, and convulsing, and two ultimately died.[552] Abruptly switching gilts to a diet containing 13,600 ppm NaCl resulted in feed refusal and watery diarrhea lasting several days.[553] Dosing an 11.3-15.9 kg (25-35 lb) pig with approximately 178.6 g NaCl (69.6 g Na^+) in 500 ml water resulted in death within 15 minutes.[554] A female dog became blind, convulsed, and eventually died after consuming seawater (26,800 mg NaCl/L) on a hot summer day.[544] Three female Boston terriers became ataxic, convulsed, and later died after drinking water contaminated with brine and containing approximately 100,000 mg NaCl/L.[555] A male Airedale terrier began having seizures and eventually died after ingesting a bolus of a salt-mixture clay. The concentration of NaCl in the clay was not reported; however, it was stated a minimum of 20 g NaCl still remained in the dog's stomach.[548] The minimum toxic dose in canines was estimated at 2,000 mg NaCl/kg BW (780 mg Na/kg BW).[556] Rats refused to drink water containing 25,000 mg NaCl/L; death occurred after several days of water refusal were followed by gorging on water.[557]

Chronic toxicity has been investigated under both field and experimental conditions. Cattle consuming water containing 12,000 mg NaCl/L (4,680 mg Na^+/L) throughout the summer months did not become clinically dehydrated, but they did exhibit diarrhea, a 28.2% reduction in feed intake, and a 69% increase in water intake.[558] Cattle ingesting water containing 2,500 mg NaCl/L (975 mg Na^+/L) for 28 days showed increased water intake, decreased milk production, and diarrhea.[547] Steers fed diets containing 50,000 ppm NaCl (19,500 ppm Na) for up to 175 days gained less weight and utilized organic matter less efficiently than controls.[531] Steers fed high grain diets containing 70,000 ppm NaCl (27,300 ppm Na) for 126 days showed increased water intake, decreased feed intakes, and altered digestion patterns.[559] Feeding lactating cows low fiber diets in conjunction with concentrate containing 60,000 ppm NaCl resulted in a 6.9% decrease in organic matter consumption and a 22% increase in water intake, but these diets had no effect on milk production.[560] Lactating heifers supplemented with 136 g NaCl/day, beginning 42 days prepartum and continuing until 10 days postpartum, showed an increased incidence of udder edema.[561] Water containing 15,000 mg NaCl/L resulted in decreased feed consumption, decreased body weight, and increased water intake in sheep.[546] Drinking water containing 10,000-13,000 mg NaCl/L resulted in neonatal mortality and distress at parturition when given to twin-bearing pregnant ewes.[562] Sheep drinking water

containing 15,000 mg NaCl/L for 21 days consumed less feed and more water than controls.[542] Sheep consuming water containing 20,000 mg NaCl/L for three days experienced a sharp decline in feed intake. After five days drinking the water, the animals' feed consumption eventually recovered, suggesting an adaptive response.[563] Lambs given 2 g NaCl/kg BW from an early age had significantly lower growth rates, reduced feed intakes, and diarrhea in comparison to controls.[564] Pigs consuming 15,000 mg NaCl/L water for 30 days became stiff legged and nervous, and they had reduced water intakes.[565] Hypertension has been produced in rats by offering water containing 10,000-25,000 mg NaCl/L for six months or longer.[566-568] Feeding trials determined the chronic oral LD_{50} of NaCl fed to rats for 100 days was 2.69 g/kg BW.[569]

Toxicity has also been shown to occur when elevated dietary NaCl is coupled with water restriction. Feeding 60 3-year-old steers a supplement containing 4.5 kg NaCl one day prior to withholding feed and water (to obtain fasting body weights) resulted in blindness, incoordination, knuckling of fetlocks, and recumbency in five animals.[543] Goats offered diets containing 30,000 ppm NaCl for 30 days showed a 20% decrease in feed intake when water was freely available and a 43% reduction when water was restricted.[570] Pigs fed NaCl at the rate of 2.4 g/kg BW with restricted water showed "extreme signs" of NaCl toxicity and ultimately died.[571] Sodium chloride poisoning was diagnosed in a herd of pigs that exhibited convulsions, an unsteady gait and muscle-twitching, blindness, and rapid breathing. The pigs had been without water for an undetermined amount of time, and no levels of dietary NaCl were given.[572] Swine began having convulsions and were exhibiting head pressing within three days of being purchased at an auction. It was determined the clinical signs developed as a result of the pigs receiving a high salt diet prior to the sale and then receiving no water during the auction.[573]

There are several reports that indicate at least some Na can be tolerated. Offering cattle water containing 4,110 mg NaCl/L resulted in increased water intake and diuresis; however, no other effects on animal health were noticed.[574] Cattle consuming water with 5,000 mg NaCl/L (2,000 mg Na^+/L) appeared clinically normal throughout the course of two 30-day experiments.[539] Steers consuming 192-193 g NaCl/day for 84 days showed no observable negative effects when ingesting a roughage diet; however, when fed a corn silage diet, the animals consuming the high Na diet had reduced weight gains and feed efficiencies. Roughage diets tend to increase water intake, potentially diluting NaCl and decreasing its effects.[532] Sheep consuming water containing 13,000 mg NaCl/L for up to 16 months increased their water intake, but they exhibited no other adverse health effects.[540,575-580] Rations with a NaCl concentration of 91,000 ppm fed to sheep caused no significant effects on breeding, gestation, lambing, or wool production.[581] Swine consuming 5,150 mg NaCl/L water had a drastic increase in water intake; however, no other adverse effects were noticed.[582]

The limited data available regarding Cl^- in water seems to indicate it is primarily a palatability factor. The EPA's secondary water standard for human consumption suggests that more than 250 mg Cl^-/L imparts a "salty" taste.[583] Water containing 5,000 mg $CaCl_2$/L offered to cattle resulted in water refusal despite lack of any other water sources. Water containing 3,000 mg/L resulted in increased water consumption and urinary acidification but no measurable effects on performance or health.[584] The SO_4^{2-} ion was less palatable to beef heifers than the Cl^- on an equimolar basis.[585] Rats drinking water containing 10,000 mg $CaCl_2$/L were unable to produce normal litters, and 15,000 mg $CaCl_2$/L reduced growth rates.[586] Conversely, in another study, rats consuming 15,000 mg $CaCl_2$/L adapted, grew normally, reproduced, and were able to suckle their young.[557]

Summary

The effects of Na and Cl are difficult to separate since neither exists in its pure state in nature, and the elements usually occur together in water. It is also difficult to separate the chronic effects of NaCl from those attributed to TDS in the literature, as the Na^+ and Cl^- ions are major constituents of salinity under natural conditions. Nevertheless, it is the Na^+ ion that seems to be responsible for most of the recognized effects of "salt" poisoning.[583] At present, there is not sufficient data to make any specific recommendations regarding Cl^- in drinking water for livestock or wildlife.

The toxic effects of Na are very dependent upon the availability of fresh water. If abundant, good quality drinking water is available, animals can tolerate large doses of Na. This fact notwithstanding, at some point excess dietary Na exceeds the ability of the organism to excrete Na^+, and acute poisoning results, regardless of water intake. The threshold for acute toxicity seems to be approximately 1g Na/kg BW for swine, with cattle equal or slightly less sensitive and sheep considerably less sensitive (roughly

2.5 g Na/kg BW). Most animals will limit their consumption of NaCl to approximately 400 mg Na/kg BW/day, if possible. Beyond this dose, feed intake, water consumption, and productivity decline. We couldn't find sufficient equine-specific data to nominate a toxic dose, but most reviewers indicate horses are roughly similar in sensitivity to cattle and swine. Similarly, there is virtually no quantitative data on wild ungulates, but there is no reason to suspect wild ruminants are significantly more sensitive than cattle.

If the only water available is also the major source of dietary Na, long-term impacts will occur at lower dosages. Chronic health effects, mainly decreased production, have been reported at water concentrations as low as 1,000 mg Na^+/L in dairy cows; however, other studies with beef heifers in cooler climates reported only minimal effects at 1,600-2,000 mg Na^+/L. Interestingly, the actual *doses* of Na consumed by the cattle in all of these studies (250-400 mg Na^+/kg BW) were similar. Dosages greater than 800 mg Na/kg BW resulted in effects ranging from weight loss and diarrhea to death.

It is theoretically possible animals maintained on high-Na^+ water for prolonged periods will, if suddenly exposed to low-Na^+ water, develop acute Na^+ ion intoxication, and anecdotal reports suggest that such has happened under field conditions. We were unable, however, to discover sufficient quantitative information to make any recommendations other than animals should be transitioned from high to low Na^+ water sources gradually.

Assuming water consumption typical of a rapidly growing steer (see Introduction) and only background feed Na concentrations, the no-effect level would be about 1,000 mg Na^+/L or 2,500 mg NaCl/L. Serious effects, including death, become likely at 5,000 mg Na^+/L. We recommend keeping drinking water Na concentrations at less than 1,000 mg/L, although short-term exposure to concentrations up to 4,000 mg/L should be well-tolerated.

10 Sulfur

Sulfur (S) occurs in nature as free S or combined with other elements in sulfides and sulfates. The most common form in water is the sulfate (SO_4^{2-}) ion, although some sulfurous wells may contain relatively high concentrations of dissolved sulfides. The latter do not persist for long under surface conditions but may contribute to health problems while they are present. Sulfides in igneous and sedimentary rock are oxidized to sulfate (SO_4) during the weathering process. The resulting sulfate salts are leached from soils by runoff and may be concentrated by evaporation in playas. Some aquifers are naturally very high in SO_4^{2-}. Once SO_4^{2-} is dissolved in water it cannot be removed unless it is reduced to sulfide by anaerobic organisms and precipitated in sediments, released as hydrogen sulfide (H_2S) or incorporated into organic matter.[587] Reverse osmosis, distillation, and ion exchange may be used to remove SO_4^{2-} from water; however, none of these processes is cost-effective for livestock under normal conditions. Sulfur may also be present in organic compounds synthesized by aquatic biota; however, this form is usually a relatively minor component of the total water S content. In 1997, 11.5% of 454 forage/water pairs collected from around the United States yielded dietary S concentrations potentially hazardous for cattle. Thirty-seven % of these elevated pairs originated from the western United States, including Wyoming.[588]

Essentiality

Sulfur is essential for health and, in fact, comprises about 0.15% of the total body in mammals, where it is a constituent of the amino acids methionine, cysteine, cystine, homocysteine, cystathionine, taurine, and cysteic acid. It is also a component of biotin, thiamin, estrogens, ergothionine, fibrinogen, heparin, chondroitin, glutathione, coenzyme A, and lipoic acid.[589] Calves deprived of dietary S had smaller livers, spleens, and testes, and larger brains and adrenals than controls.[590] Lactating dairy cows require between 0.17% and 0.20% total dietary S to remain in positive balance, as S constitutes an estimated 0.78% of milk proteins.[591] The nutritional S requirement of monogastric mammals must be provided as two amino acids – methionine and cystine. Ruminants can use either preformed amino acids or synthesize S-amino acids from inorganic S; however, the efficiency of the latter process varies with other dietary conditions.[592-594]

Metabolism

The first step in ruminal synthesis of S-amino acids from inorganic S is reduction of the latter to H_2S.[595] Not surprisingly, SO_4 is converted to H_2S more efficiently than pre-formed S-amino acids. Halverson et al.[596] examined sulfide production from various S sources and found methionine produced one third the amount of sulfide as SO_4. Under normal circumstances, the reactive sulfide ion is combined with carbon by rumen microflora to create methionine, homocysteine, cystathionine, cysteine, and other S-amino acids. Under conditions of excessive S intake, however, significant quantities are reduced to H_2S, and the very toxic gas escapes from the rumen into the systemic circulation resulting in poisoning.[597-601]

Excess rumen sulfide may also interact with certain trace elements, especially Cu, decreasing bioavailability and possibly resulting in serious nutritional deficiencies.[241,447,602-604] In ruminants, S combines with Mo to form thiomolybdates. These, in turn, form unabsorbable complexes with Cu, which irreversibly bind to the solid phase of the digesta, resulting in Cu deficiency.[241] It has also been suggested that thiomolybdates interfere postabsorptively with Cu incorporation into the enzymes superoxide dismutase and cytochrome oxidase, compromising mitochondrial integrity and cell function.[230,241,605] Finally, it has been theorized that some of the effects of excess dietary Mo are actually due to Mo toxicity *per se* and not to hypocupremia.[241] Of the three elements, Cu, Mo, and S, S provides the most variation in nutritional outcomes due to its multiple metabolic pathways from the rumen. Sulfur exits the rumen principally as sulfide, but it can also leave as undegraded protein S or be incorporated into microbial protein. Inorganic S from diet, saliva, or degraded protein is the only form of S that will interact with Mo and Cu. Several factors affect protein degradation to S, including the supply of degradable nitrogen, the rate of ingestion, the specific population of rumen microbes, and the availability of readily fermentable carbohydrates.[241]

Dietary S may also antagonize Cu metabolism in the absence of excessive Mo. Copper deficiencies occurred in cattle fed 0.3% total dietary S[606], and 500 mg S/L water was hypothesized to cause secondary deficiency as it raised dietary S to 0.35%, both in the absence of excess Mo.[230] Sulfur inhibits the uptake of Zn. The interaction between Zn and S is further magnified if animals are fed a high fiber diet.[603] Sulfur also decreases the uptake of dietary Se.[445,607] High dietary concentrations of S are thought to reduce rumen pH, favoring the conversion of Se to biologically unavailable selenide.[445,608] Sulfur may also reduce incorporation of dietary Se into ruminal bacterial protein.[447] Interestingly, S has been shown to protect against Se intoxication under some circumstances.[445,448]

Monogastric animals lack the ability to produce S-amino acids from inorganic S and are thus somewhat less sensitive to the above-mentioned toxic effects of the sulfide ion. For example, the NRC "maximum tolerable" S concentration for range cattle is 0.5%, whereas 0.69% of diet is *optimal* in rats.[589] Although it is possible for a monogastric to generate toxic concentrations of H_2S following ingestion of elemental S, the dosage required is much greater than in ruminants. To illustrate this, 14 horses were mistakenly fed between 0.2-0.4 kg of flowers of S (99% S) resulting in a dose between 333-666 mg/kg BW (corresponds to 11-22% dietary dry matter). The horses became ill within 12 hours, and two died after 48 hours. *Post mortem* examination of the two deceased animals revealed cranioventral consolidation of the lungs, hemorrhaging throughout the heart and GI, and congestion of the liver.[609] Toxic effect(s) of inorganic S salts (e.g. SO_4) in monogastric species are usually related to abnormalities in water balance in the GI tract, explaining why clinical signs differ between monogastric and ruminant mammals. In swine, toxic effects are generally manifested as watery feces and have been shown to occur when ingesting water with concentrations as low as 600 mg SO_4^{2-}/L, but they more commonly occur in water containing 1,600 mg SO_4/L or higher.[610-612]

Toxicity

As with all poisons, toxicity depends on dose, route of exposure, and form of the element. In this report, we are most interested in ingestion (oral exposure) and SO_4^{2-}, as that is the form of S commonly found dissolved in water. Between 0.3-0.5% of dietary dry matter is the recommended maximum tolerable limit for total daily S intake for ruminant animals.[589] The amount of S that water contributes to the diet depends on the amount of water an animal drinks as well as the concentration of S in the water. This varies drastically with environmental temperature, type of feed, and condition of the animal. In one published example, the amount of S contributed by 1,000 mg SO_4^{2-}/L in drinking water varied from 0.1-0.27% under different conditions.[613]

Toxic S concentrations have been shown to reduce the feed intake, water intake, growth, and performance of animals. Cattle given water containing 1,219 mg SO_4^{2-}/L in conjunction with a diet containing 0.16% S (0.29% total S intake), exhibited depressed dry matter intake (DMI).[614] Adding 0.72% SO_4 (0.24% dietary S) to cattle diets reduced weight gains by 50% after the first two weeks.[615] Concentrations of 0.35% or more dietary S resulted in diminished DMI in lactating dairy cows.[591] Water containing 5,000 mg sodium sulfate (Na_2SO_4)/L and grass hay containing "0.75% SO_4" reduced water intake by 35% and feed intake by 30% in cattle.[574] Decreases in average daily gain (ADG), feed efficiency, and dietary net energy were seen when heifers were fed 0.25% S as ammonium sulfate ($(NH_4)_2SO_4$).[616] Supplying heifers with water containing 2,814 mg SO_4/L and hay containing "0.55% SO_4" reduced hay intake by 12.4% during the summer months.[617] Water containing 3,087 mg SO_4/L reduced ADG by 27%, DMI by 6.2%, and water intake by 6.1 L in steers, and it increased the incidence of polioencephalomalacia (PEM).[618] Cattle on a low plane of nutrition decreased their water intake when consuming water with 1,000 mg SO_4/L, and cattle on a high plane of nutrition had a slight decrease in feed intake when consuming 2,000 mg SO_4/L.[619] Concentrations of S greater than 0.4%, added as elemental S or Na_2SO_4, decreased gains in feeder lambs.[594] Approximately 0.5% S added to rations as calcium sulfate ($CaSO_4$) or Na_2SO_4 resulted in reduced feed intake and daily gains of 163 g/day and 191 g/day, respectively, compared to control lambs that gained 251 g/day.[620]

On the other hand, when 0.75% S was added as $CaSO_4$ to the concentrate portion of the diet (0.477% total dietary S) of six Hereford cattle, no statistically significant changes in serum enzyme activity, Se concentrations, weight gains, or general health were noticed.[621] The study, however, was designed to look at S-Se interactions and lacked statistical power to examine growth, ADG, or other measures of performance, and one of the animals died of PEM at the end of the experiment. Cattle offered water containing 2,500 mg SO_4/L showed no changes

in feed or water consumption. The animals consumed an average of 3.9 kg of hay, 3.1 kg concentrate, and 33.1 kg water per day, suggesting this level as a safe tolerable limit.[585] As with the previous study, the number of animals tested was very small. Sublimed S added to the diet of steers for 10 weeks at 0.42% did not affect feed intake but did at 0.98%.[622] Qi et al.[623] added various amounts of $CaSO_4$ to the diet of goats and concluded that optimum feed performance occurred between 0.2-0.28% S.[623] Pendlum et al.[624] fed up to 0.3% elemental S to steers without adverse effects.

The most dramatic manifestations of S toxicity in ruminants are sudden death, with no lesions, and/or PEM. Polioencephalomalacia is a neurological disease of cattle and sheep, resulting in seizures, ataxia, blindness, and recumbency as the main clinical signs. It is usually fatal. Seven hundred of 2,200 ewes grazing a pasture previously sprayed with elemental S began showing signs of abdominal discomfort within two hours of exposure, and 220 ewes died within five days. Lesions of PEM were found only in the sheep that had survived for five days.[625] Animals ingesting water with 4,564 mg SO_4/L and feed containing 0.17% S had a 47.6% incidence of PEM and a 33% mortality rate.[626] Six of 110 cows drinking 7,200 mg Na_2SO_4/L water developed PEM.[627] Eighteen of 21 herds fed supplements containing 2% inorganic SO_4 developed PEM. This supplement provided approximately 0.16% S beyond what was in the rest of the diet.[628] Water containing 2,000 mg SO_4^{2-}/L produced PEM in one of nine steers.[597] Three of 21 steers fed 3,780 mg SO_4^{2-}/L developed PEM and died. Feeding thiamin did not prevent S-toxicity.[629] Four steers died of PEM on a feedlot in Alberta, Canada, after consuming water with 5,203 mg SO_4/L while the temperature was 30 C.[630] Four of 40 animals developed PEM after ingesting hay with 0.39% S and water containing 2,250 mg SO_4/L.[631] All 10 experimental animals offered water with 5,540 mg S/L or 7,010 mg S/L showed signs of PEM.[632] The incidence of death from PEM in a beef feedlot varied dramatically with environmental temperature, from none in the winter to 0.8% per month in the summer. The increase also corresponded with a 2.4-fold increase in water intake as a result of summer weather, raising total dietary S intake to 0.67%.[633] Cows in Canada were stricken with PEM when exposed to 3,400 mg SO_4^{2-}/L water; no new cases occurred after the water was replaced.[634] Sixty-nine animals were affected with PEM after ingesting a protein supplement containing "1.5% organosulfate" and water containing 1,814 mg SO_4/L.[205] Experimentally feeding 0.477% total dietary S resulted in one of 12 heifers developing PEM three days after termination of the experiment.[621] Polioencephalomalacia has been diagnosed in wild ruminants.[635,636] Tests were not conducted to confirm SO_4 as the cause of these cases, but surface waters in the area where the animals were found are naturally high in SO_4, and exposure to these waters was considered likely.[637]

Sulfate waters are quite unpalatable, and, when given a choice, animals will discriminate against them. A taste test was conducted between waters containing 1,450 mg/L and 2,150 mg/L SO_4 and tap water. The cattle discriminated against the water containing 1,450 mg/L and rejected the water containing 2,150 mg/L, opting for tap water instead.[617] Despite the unpalatability, if no other water is available, animals will reluctantly drink water with higher SO_4 concentrations resulting in potential toxicity.

Summary

In ruminants, high dietary S may cause acute death, PEM, trace mineral (especially Cu) deficiencies, and/or chronic, as-yet-poorly-defined ailments that decrease production efficiency. All dietary sources of S (water, forage, concentrates, feed supplements) contribute to total S intake and thus to potential toxicity. The S contribution of water, usually as the SO_4^{2-} ion, varies dramatically with environmental conditions as water consumption goes up and down.

From a strictly theoretical standpoint, the NRC maximum tolerable dose of S for cattle is 0.5% of the total diet (0.3% for feedlot animals).[589] Wyoming grasses are reported to contain between 0.13%-0.48% S.[638] Assuming forage S concentrations of 0.2% and water consumption typical of young, rapidly growing cattle at summer temperatures (30 C), a water SO_4 concentration of 1,125 mg/L will meet or exceed the NRC's maximum tolerance limit for S in cattle. Adult bulls, which consume half as much water, could theoretically be impacted by 2,250 mg/L, and lactating cows would fall somewhere in between. In practice, water SO_4 concentrations as low as 2,000 mg/L have caused PEM and/or sudden death in cattle. This observation is supported by many field cases investigated by the WSVL and other regional diagnostic labs since 1988. It *seems* to be contradicted by some of the early studies mentioned above, notably Digesti and Weeth[585], but both probability and the morbidity of poisoning increase with progressively larger SO_4 concentra-

tions; thus, studies with small numbers of animals easily overlook marginally toxic doses. Anecdotal data also indicate cattle are able to adapt to elevated S concentrations *if* introduced gradually to potentially toxic waters over a period of several days to weeks. The details (i.e. how rapidly dietary S can change) of this process and the effect(s) of other dietary factors such as energy and protein on the process are still a matter of conjecture.

Waterborne SO_4 is reported to decrease Cu uptake at concentrations as low as 500 mg S/L as SO_4^{2-}.[602,606] Whether overt Cu deficiency results depends upon the dietary concentration of Cu, and excess dietary Cu may compensate for some or all of the effect of SO_4^{2-}.[308] Unfortunately, most Wyoming forages are marginally to drastically deficient in Cu for cattle. Elevated dietary S also interferes with the uptake of Zn and Se. Trace element deficiencies are multifactorial diseases that do not normally manifest themselves unless animals are exposed to other stressors such as bacterial pathogens, bad weather, shipping, etc. Therefore, it is difficult, if not impossible, to settle upon a single number that consistently results in deficiency or guarantees safety; however, the NRC recommends "the sulfur content of cattle diets be limited to the requirement of the animal, which is 0.2% dietary sulfur for dairy and 0.15% in beef cattle and other ruminants."[589]

Relatively low S concentrations (equivalent to 500-1,500 mg SO_4^{2-}/L in water) have also impacted performance (e.g. ADG, feed efficiency) in feedlot and range cattle via a variety of mechanisms not completely understood.[614,616,639,640] Loneragan et al.[597] suggested that H_2S produced from SO_4^{2-}, eructated and then inhaled, resulted in pulmonary damage and increased susceptibility to respiratory infections. Elevated SO_4^{2-} also results in decreased water intake under experimental conditions. Finally, it is possible some, as yet unrecognized, interactions with other dietary components result in decreased utilization and feed efficiency. These effects have obvious implications for animal health, but they are difficult to quantify under field conditions.

Monogastrics, such as horses, are at less risk of S effects that involve ruminal generation of sulfide. In these species, the principle effect of elevated drinking water SO_4 seems to be diarrhea resulting from the osmotic attraction of water into the gut. The relative contributions of the SO_4^{2-} ion and its associated cation are unclear, but the literature indicates the effect 1) is transient and not life-threatening and 2) probably only occurs at concentrations considerably in excess of those toxic in ruminants. Therefore, concentrations that are safe in ruminants should provide adequate protection for horses.

Assuming normal feedstuff S concentrations, keeping water SO_4^{2-} concentrations less than 1,800 mg/L should minimize the possibility of acute death in cattle. Concentrations less than 1,000 mg/L should not result in any easily measured loss in performance.

11 Total Dissolved Solids (TDS)

Total dissolved solids (TDS) is defined as all inorganic and organic substances contained in water that can pass through a 2 micron filter. In general, TDS is the sum of the cations and anions in water. Ions and ionic compounds making up TDS *usually* include carbonate, bicarbonate, chloride, fluoride, sulfate, phosphate, nitrate, calcium, magnesium, sodium, and potassium, but *any* ion that is present will contribute to the total. The organic ions include pollutants, herbicides, and hydrocarbons. In addition, soil organic matter compounds such as humic/fulvic acids are also included in TDS. There are a variety of ways to measure TDS. The simplest is to filter the water sample, and then evaporate it at 180° C in a pre-weighed dish until the weight of the dish no longer changes. The increase in weight of the dish represents the TDS, and it is reported in mg/L. The TDS of a water sample can also be estimated fairly accurately from the electrical conductivity of the sample via a linear correlation equation dependent upon specific conductivity. Finally, TDS can be calculated by measuring individual ions and simply adding them together.

Total dissolved solids is a non-specific, quantitative measure of the amount of dissolved inorganic chemicals but does not tell us anything about **its** nature. TDS is not considered a primary pollutant with any associated health effects in human drinking water standards, but it is rather used as an indication of aesthetic characteristics of drinking water and as a broad indicator of an array of chemical contaminants.

Essentiality

Although many essential elements may contribute to TDS, the measurement technique does not, itself, differentiate essential from toxic elements.

Metabolism

Since TDS represents an undifferentiated collection of just about everything dissolved in a water sample, it is impossible to speak of the "metabolism" of TDS.

Toxicity

Interestingly, early epidemiologic studies suggested that "moderately high" TDS concentrations ("high" in this context being less than 1,000 mg/L) protected people against cancer and heart disease.[641-644] Although the mechanism(s) underlying these early observations are not completely understood, it was first narrowed down to "hardness" as opposed to TDS. It now appears certain constituents of TDS, notably Mg, interfere with the formation of thrombi in arteriosclerosis.[641,645] Another hypothesis for the protective effect is that some components of hardness decrease leaching of toxic elements from plumbing.[644] The inclusion of other cardiac risk factors, such as Na, in the total TDS of earlier studies probably accounts for the conflicting results in the older literature.

Saline waters may adversely impact animal health by several possible mechanisms. One of the most important biological functions of water in mammals is as a solvent for nutrients, waste products, etc. The presence of extraneous solutes decreases the ability of water to serve this function by decreasing its ability to dissolve additional solutes. A similar, related factor is plasma osmolarity. Solutes exert an attraction on water across membranes, and inappropriate water movement is disastrous to cells and tissues. An extreme example of this effect is water intoxication that results in death, as was the case with a young woman in California.[646] Mammals expend a considerable amount of energy maintaining the osmolar concentration of various body compartments within a fairly narrow range. The presence of excessive solutes in drinking water adds to this burden and consumes resources that would otherwise be used for growth, milk production, or fighting off disease. It is well-accepted that extreme drinking water TDS concentrations in the 1.5%-3% range are incompatible with life[546,551,557,586,647,648]; however, the effects of lower TDS concentrations are too multifactorial, involving species, age, sex, diet, pregnancy, lactation, environmental conditions, etc., to lend themselves to simple all-or-nothing results. Also, the fact animals may "tolerate" (in other words, survive) a particular concentration is not the same as proving they remained productive on it.

Elevated TDS adversely affects the palatability of water. In humans, taste panels rated the palatability of water with 300 mg/L as "excellent," 300-600 mg/L "good," 600-900 mg/L "fair," 900-1,200 mg/L "poor," and greater than 1,200 mg/L "unusable." Earlier criteria for human health were based upon this fact.[649] In livestock, decreased palatability is well-recognized as an important determinant of water consumption and, indirectly, feed consumption and performance. Cattle given water containing 6,000-15,000 mg/L TDS exhibited decreased water intake, feed intake, and average daily gain (ADG).[550,618,626,650-652] Five thousand mg/L decreased feed intake and gain of cattle on a high roughage diet.[653] Dairy cows given 2,040 mg/L water consumed less water and produced less milk when the peak ambient temperature was 32.1 C than cows given desalinated water.[654] Similar decreases in milk production attributed to consuming saline water were seen in Arizona.[547,655] Swine subsisting on water containing 10,000-15,000 mg/L drank less, ate less, and performed more poorly than controls.[565] Sheep seem to be more tolerant of saline waters than most domestic species and will drink them if introduced to the saline water over a period of several weeks.[540,541,575-580,656,657] The two references regarding saline waters in horses indicate they are reluctant to drink such water[551], and it has been alluded they can be maintained on water containing up to 9,500 mg/L TDS.[658] Limited studies with farmed deer in Australia indicate TDS concentrations as high as 4,000-6,000 mg/L are tolerated without any reduction in feed or water consumption.[659,660] We were unable to find any reports addressing the effects of salinity on wild deer.

Even when animals drink more in an attempt to compensate for poor water quality, the increased metabolic load imposed by high solute water may result in impaired performance. Water containing 1.5% NaCl (15,000 ppm) and given to cattle for less than a week resulted in a 13.7% reduction in weight, as well as decreasing feed and water-intake, and marked hypernatremia.[550] In a similar, short-term experiment at cool temperatures, cattle given 15,000 mg/L TDS water drank more, ate and grew less, and showed clinical signs of dehydration.[558] Five-thousand mg/L TDS for 51 days decreased gain in heifers.[651]

Summary

Total dissolved solids in drinking water serves as a very poor predictor of animal health. As noted above, TDS is a measure of all inorganic and organic substances dissolved in water. These individual solutes range in toxicity from relatively non-toxic substances, such as Ca^{2+}, to extremely toxic (Hg^{2+}, Se^{+4}), but tests of TDS do not differentiate between them. Several early studies suggest no significant effects in sheep at TDS concentrations up to 13,000 mg/L or cattle and swine up to 5,000 mg/L, and the NRC[661] accepted larger concentrations as tolerable "for older ruminants and horses," yet the authors have seen animals poisoned by waters in which the TDS was measured as slightly less than 500 mg/L,[415,662] and there are reports of decreased productivity in dairy cattle at 2,000-2,500 mg/L. Early epidemiologic studies in people suggested high drinking water TDS decreased the incidence of cancer and heart disease in people. Later, however, studies narrowed the active component of TDS that was negatively correlated with heart disease, first to hardness, then finally to the Mg^{2+} ion concentration. In human health, the World Health Organization dropped health-based recommendations for TDS in 1993, instead retaining 1,000 mg/L as a secondary standard for "organoleptic purposes." *The test is just too non-specific to be reliable.* As noted by Chapman et al.[663], in a study of aquatic toxicity, "Toxicity related to these ions is due to the specific combination and concentration of ions and is not predictable from TDS concentrations."

***We do not recommend relying upon TDS to evaluate water quality for livestock and wildlife;* however, if no other information is available, TDS concentrations less than 500 mg/L should ensure safety from almost all inorganic constituents. Above 500 mg/L, the individual constituents contributing to TDS should be identified, quantified, and evaluated.**

12 Summary

Element	Short (days – weeks) Exposure	Chronic (months) Exposure	Rationale
Arsenic	1 mg/L	1 mg/L	Does not seem to be a carcinogen in livestock; therefore, a concentration that protects against cytotoxic effects should be safe (pg 8).
Barium	See text page 13	No recommendation	Until there is better data we cannot make any firm recommendation regarding Barium. See text for interim suggestions (pg 13).
Fluoride	2 mg/L	2 mg/L	Prevents dental lesions in most sensitive life stage. Fully mature animals may be able to tolerate more (pg 18).
Molybdenum	0.3 mg/L	0.3 mg/L	Prevents secondary Cu deficiency and poor performance (pg 24).
Nitrate	500 mg/L	500 mg/L	Prevents acute death and abortion in well-managed cattle. Dry diets high in NO_3 may require lower concentrations (pg 28).
Nitrite	100 mg/L	100 mg/L	Prevents acute death and abortion in well-managed cattle. Dry diets high in NO_3 may require lower concentrations (pg 28).
pH	No recommendation	No recommendation	There is considerable evidence that animals tolerate a much wider range than the commonly cited 6.5-8.5, but we could not find sufficient information to make specific recommendations (pg 32).
Selenium	0.1 mg/L	0.1 mg/L	Prevents selenosis in equidae. Can probably tolerate slightly high concentrations for very short periods (pg 38).
Sodium	4,000 mg/L	1,000 mg/L	Assuming normal feedstuff Na concentration and no other water sources, these concentrations should protect against acute lethality or chronically, poor performance (pg 43).
Sulfate	1,800 mg/L	1,000 mg/L	Assuming normal feedstuff S concentration, acute death may occur in ruminants at concentrations greater than 2,000, *especially if not allowed time to acclimate*. Long-term consumption result in poor performance (pg 47).
TDS	No recommendation	No recommendation	We do not recommend relying upon TDS to evaluate water quality for livestock and wildlife (pg 50).

13 Research Needs

pH – The original acceptable range often cited in various extension and regulatory documents was designed to protect plumbing rather than health. Research suggests rodents and swine can not only tolerate but actually thrive on significantly lower pH activities than current standards. There is some indirect evidence from the dairy industry, where acidogenic diets have been used therapeutically, that cattle (and probably other ruminants) should perform well on moderately acidic water for months at a time. There is nothing to indicate what, if any, limits should be for long-term consumption. Nor is there any experimental evidence to indicate even the mild alkaline pH activity recommended under current guidelines, let alone stronger bases, are safe for animals. It might not be possible to devise a simple guideline that fits all situations as the acid-base status of mammals is very multi-factorial, but it should be possible to at least determine a range at which the direct, local effects of alkaline waters are likely to cause refusal.

Ba – The existing human guidelines are predicated upon the theoretical potential for the Ba^{2+} ion to exacerbate chronic cardiovascular problems. The existing knowledge base regarding Ba toxicity in livestock and wildlife is exceedingly scant and seemingly contradictory. If, as seems likely, large ungulates respond similarly to Ba as humans and rodents, toxicity could potentially occur at similar water concentrations. Large-scale, chronic experiments of the sort required to conclusively establish a chronic NOAEL will be very expensive. It might be more cost effective to elucidate the comparative toxicodynamics (bioavailability from various sources, pharmacokinetics, pharmacodynamics) of Ba in major livestock and wildlife species vs. rodents with an eye to extrapolating the large human/rodent database to these species.

Wildlife – There is a surprising deficit of quantitative toxicologic data in big game wildlife species, especially when one considers the resources that have been lavished on fish and insects for the last three decades. Given the physiological similarities to domestic livestock, it should not be necessary to reinvent the complete knowledge base. Relatively simple comparative studies of basic toxicologic parameters such as bioavailability between (e.g.) mule deer and domestic sheep would allow extrapolation from the existing knowledge base in livestock to wildlife.

Nitrite – While there is substantial anecdotal evidence NO_2 is more toxic than NO_3, especially in non-ruminants, we didn't find a great deal of quantitative dose-response data about oral exposure in livestock or wildlife. What we did find suggests a maximum tolerated dose considerably higher than the NRC.[2] The latter recommended keeping NO_2 extra low to compensate for NO_2 formation in slurried feedstuffs to swine. This practice is no longer common, nor is the caveat appropriate to range conditions. A reliable maximum tolerated dose, appropriate to Western range conditions, is desirable.

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